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September 1985
Volume 69
Number 9
Pages 933–1038

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1. Authors should discuss briefly the quality control methods used to ensure that the data are complete and accurate. A reliable procedure should be cited for ensuring that all patients entered on study are actually reported upon. If no such procedures are in place, their absence should be noted. Any procedures employed to ensure that assessment of major endpoints is reliable should be mentioned (eg, second-party review of responses) or their absence noted.

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3. The study should not have an inevaluability rate for major endpoints of greater than 15%. Not more than 15% of eligible patients should be lost to follow-up or considered inevaluable for response due to early death, protocol violation, missing information, etc.

4. In randomized studies, the report should include a comparison of survival and/or other major endpoints for all eligible patients as randomized, that is, with no exclusions other than those not meeting eligibility criteria.

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9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

**From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1–3, 1985).

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1037 ANNOUNCEMENTS

Improved Control in Advanced Head and Neck Cancer With Simultaneous Radiation and Cisplatin/5-FU Chemotherapy[1]

Samuel G. Taylor, IV,* Anantha K. Murthy, John L. Showel, David D. Caldarelli, James C. Hutchinson, Jr, Lauren D. Holinger, Toby Kramer, and Krystyna Kiel[2]

Chemotherapy with cisplatin plus 5-FU infusion was given simultaneously with radiation therapy; both were administered every other week. Twenty-seven patients with stage IV disease and three with stage III disease were treated; 57% had T4, 33% had N3, and 10% had M1 disease. Of these patients, 17 (57%) achieved complete response and the rest achieved partial response. However, whether or not a clinically complete response occurred had no effect on subsequent risk for disease recurrence. With a median follow-up of 16 months (range, 6–35), only 11 patients (37%) have had disease recurrence and eight (27%) have died. Of those patients who recurred, six failed in distant sites only, one developed a second head and neck primary, and four (36% of all failures) failed regionally. Normally, > 50% of patients who are initially controlled (usually < 60% of stage IV patients) would have failed within the first 12 months and ≥ 75% of these would be expected to fail regionally. Nine patients who had received previous treatment or who had M1 disease accounted for six of the recurrences and four of the deaths. Because of the excellent local control, we have cautiously limited surgery to conserve laryngeal, tongue, and mandibular function in selected cases. Neither immediate nor long-term toxicity from radiation appeared increased. Although no patient with such advanced disease can be considered cured at this time, these results seem to represent a substantial improvement in the quality of life and degree of local control over other approaches. Controlled trials of the sequencing of radiation and chemotherapy in head and neck cancer appear indicated. [Cancer Treat Rep 69:933–939, 1985]

Therapy of stage III and IV cancers of the upper aerodigestive systems has remained largely ineffective, despite the use of more radical surgery and combination chemotherapy adjuvants. Adjuvant chemotherapy programs having clinically complete response rates as high as 35% have failed to alter the subsequent rate and pattern of recurrence and disease course when tested in randomized controlled clinical trials (1–4). Of "inoperable" patients treated with primary radiation therapy, plus or minus subsequent surgery depending on response, 55% achieve complete responses, but 50% of these responders will recur in the first year. Median survival of all patients has usually been < 1 year with such advanced cancers.

In 1981, we began to use a treatment approach based on the work of two groups of investigators. Chan et al (5) have described the synergistic interaction of 5-FU infusion plus radiation delivered together every other week. They had treated 18 patients with head and neck cancer with this regimen, and reported that 56% responded with complete tumor clearance and 53% survived with a follow-up of approximately 2 years (6). Weaver et al (7) had noted a synergistic interaction between cisplatin and 5-FU infusion and reported the highest complete response rate of any drug combination used in patients with previously untreated head and neck cancer. They reported a complete response rate of 55% to the chemotherapy alone, and, with subsequent local treatment, achieved a median survival of 66 weeks (8).

In addition, based on animal data (9,10), a number of investigators have reported synergy between cisplatin and radiation therapy (11–14). Complete response rates in these studies ranged from 61% to 86%, but < 50% of patients remained disease-free by 18 months. 5-FU has also demonstrated synergy with radiation in one of the

[1]Received Aug 31, 1983; revised Feb 22, 1985; accepted Mar 6, 1985.

[2]Departments of Medicine (*S. G. Taylor, IV,* and *J. L. Showel*), Radiation Therapy (*A. K. Murthy, T. Kramer,* and *K. Kiel*), and Otolaryngology (*D. D. Caldarelli, J. C. Hutchinson, Jr,* and *L. D. Holinger*), Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL.

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few randomized trials in head and neck cancer that has yielded positive results (15). Despite these innovative approaches, most head and neck cancers recur regionally, and most recur within the first year of follow-up.

We have used these concepts to devise a treatment program that we have used initially only for patients with very advanced head and neck cancer beyond the hope of cure with current definitive local therapies.

METHODS

Initially, patients were selected for study if they had histologically confirmed carcinoma of the upper aerodigestive tract that was considered incurable with standard radiation therapy and surgical techniques and if the creatinine clearance was > 50 ml/minute. Although patients previously treated with radiation therapy were not routinely considered for this protocol, four patients who had received between 45 and 60 Gy were treated with a limited number of cycles using this combined modality. Another patient had received 65 Gy to a laryngeal primary. Since the irradiated area had been resected, she was able to receive six cycles of therapy. Because of the striking responses seen in the first 12 patients, the criteria for entry were subsequently enlarged to include patients with earlier stage IV disease and a few with stage III disease, who might otherwise have required mutilating radical surgery.

Treatment with radiation and chemotherapy was given concomitantly on an every-other-week schedule, as shown in figure 1. Cisplatin was administered on Day 1 following hydration with 1000 ml of 5% dextrose in one-half normal saline over 2 hours and 12.5 g of mannitol by iv push. After cisplatin, 1000 ml of normal saline with 40 g of mannitol was given over 4 hours and then 2000 ml of 5% dextrose in one-half normal saline with 40 mEq of KCl over 8 hours. Frequent problems with aggravation of facial edema with this hydration program have led to substituting furosemide for the mannitol. Patients usually received flurazepam (Dalmane) (60 mg orally) and meclorpropamide (2 mg/kg iv) prior to cisplatin as antiemetics. The meclorpropamide was continued for three to six doses after the cisplatin, depending on the degree of nausea and vomiting. 5-FU was infused beginning immediately after the cisplatin at a dose of 800 mg/m^2 daily over 24 hours for 5 days (120 hours). No allopurinol was given at any time to any patient during this therapy.

Radiation therapy was delivered by super-voltage equipment with ^{60}Co or a 4-meV linear accelerator at source-to-axis distance of 80 cm. The areas treated included all the areas of known gross disease except in one patient with pulmonary metastases. Prophylactic irradiation to the uninvolved areas in the neck was attempted in all patients who had not received previous radiation therapy. For previously untreated patients the volumes and techniques of treatment followed those of conventional regimens. The dose used with these patients was 70 Gy over seven courses in 13 weeks, given at 2-Gy fractions, on Days 1-5 during each course.

One patient also received brachytherapy with an interstitial ^{192}Ir implant to the tongue primary. The brachytherapy was not combined with chemotherapy. Total doses were limited to 60 Gy in two previously untreated patients because of planned surgery in one and presence of pulmonary metastasis in the other.

Dosage was adjusted on the basis of mucous membrane and hematologic toxic effects. If mucosal erythema or resolving, nonpainful ulcers were present at the start of each cycle, the 5-FU dose was reduced by 25% for that cycle; if scattered painful ulcers were present, the dose was reduced by 50%, while cisplatin and radiation doses were kept at 100%. If confluent ulcers were present, all therapy was delayed by 1 week. If painful ulcers occurred between cycles, the 5-FU dose was reduced by 25%, or by 50% if confluent mucositis occurred at that time. The dose of cisplatin and 5-FU, but not radiation, was reduced by 25% for a wbc count between 2500 and 3499/mm^3 or a platelet count between 75,000 and 100,000/mm^3; it was reduced by 50% for a wbc count between 2000 and 2499/mm^3 or a platelet count between 60,000 and 74,999/mm^3. Counts lower than this led to withholding all treatment 1 week for recovery.

All patients underwent direct endoscopy for staging and were staged on the basis of the American Joint Committee (AJC) criteria of 1978 (16). Complete response was defined as the complete disappearance of all disease with normalization of roentgenograms, computerized tomographic scans, and other tests that reflect disease status. Partial response was defined as a reduction in size of > 50% for both the primary and nodal disease by comparing the product of each mass' longest diameter and its perpendicular before and during treatment. Determination of complete response was made 6 weeks after completion of radiation therapy or just prior to any surgery. Performance status and toxicity criteria are those of the Eastern Cooperative Oncology Group (ECOG) (17).

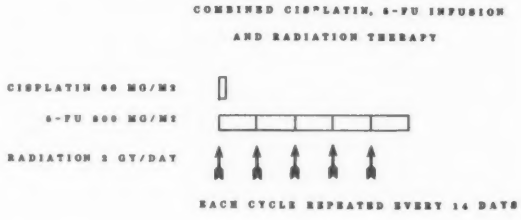

FIGURE 1.—Treatment program for combined radiation and cisplatin + 5-FU infusion for treatment of head and neck cancer. Each cycle was repeated every other wk, for a total of 7 cycles (70 Gy) in 13 wks.

RESULTS

Thirty patients were entered between July 1981 and January 1984. Patient characteristics are shown in table 1. Compared to most other series, this population had a larger proportion of patients with a performance status of 2 or worse, poorly differentiated tumors, and hypopharyngeal lesions; this reflects the advanced stage of disease in these patients. The distribution of TNM stages is shown in figure 2. One-third of the patients had N3 disease and 57% had T4 disease. One patient with T4N0 disease had bilateral pulmonary metastases and was the only patient with tumor outside the radiation fields. Two patients, with T2Nx and T4N1 disease respectively, had multiple skin nodules in the neck, indicating lymphangitic spread. These nodules are categorized as M1 disease in this report, but are not truly classifiable in either the N or M category of the AJC. In both patients, all evident disease was encompassed within the radiation fields.

Results are based on a median follow-up of 16 months (range, 6–35). Complete resolution of disease, defined as normalization of all clinical parameters and computerized tomographic scans, occurred in 17 patients (57%). Twelve of the other 13 patients did not show normalization of their clinical examinations but had tumor shrinkage of at least 75%. The worst response was in a patient with an 11 × 8-cm neck mass; it shrunk by only 50%, yet at surgery no viable tumor could be located in the resected specimen.

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Mean age in yrs (range): 63 (49–83)	
Females	8 (27)
Prior treatment	8 (27)
ECOG performance status	
0	5 (17)
1	14 (47)
2	10 (33)
3	1 (3)
Histology	
Well differentiated	2 (7)
Moderately well differentiated	16 (53)
Poorly differentiated	12 (40)
Disease sites	
Oral cavity	7 (23)
Oropharynx	3 (10)
Nasopharynx	2 (7)
Hypopharynx	9 (30)
Larynx	5 (17)
Sinus	2 (7)
Unknown	2 (7)

Table 2 gives the percentage of patients remaining relapse-free and the median times to failure of patients who had recurrence, based on various disease characteristics. Two patients who died during treatment are omitted from this analysis. Both patients had achieved complete

STAGE (AJC 1978)

NODAL STATUS	TUMOR STATUS 0	1	2	3	4	TOTAL
0			1^{MR}	1	6^{MR}	8
1				2	6^{MR}	8
2		1			3^{R}	4
3	$4^{R,R}$	1		$3^{R,R}$	2	10
TOTAL	4	2	1	6	17	30

M = ONE PATIENT WITH M1 DISEASE.

R = ONE PATIENT WHO FAILED PRIOR TREATMENT.

FIGURE 2.—Distribution of patients within each T and N category based on the staging system of the AJC (1978). Individual patients who had received prior treatment are indicated by an "R" for recurrence and those with metastatic disease by an "M."

TABLE 2.—% of patients relapse-free and time to failure based on disease characteristics

	% relapse-free	Median time to failure (mos)*
Clinical response to treatment		
Complete (n = 15)†	56	9
Not complete (n = 13)	67	7
Surgical treatment		
None (n = 19)†	58	8
Some (n = 9)	67	18, 21, 29
Surgical pathology		
No tumor (n = 6)	67	18, 29
Tumor present (n = 5)	60	7, 21
Performance status		
0 (n = 5)	60	5, 18
1 (n = 14)	71	7
2, 3 (n = 9)†	44	9
Histologic grade		
1, 2 (n = 17)†	53	9
3 (n = 11)†	73	5, 6, 18
5-FU dosage		
< 85% (n = 15)†	60	8
≥ 85% (n = 13)†	62	8
Cisplatin dosage		
< 85% (n = 9)	44	9
≥ 85% (n = 12)†	67	15

*Time to failure given as median if > 3 patients in that group failed.
†Indicates groups from which the 2 patients who died during treatment are deleted.

clinical response prior to death. Whether or not the clinical response to treatment was complete tumor clearance did not affect the risk of recurrence. Surgery was performed on nine patients and an additional two had biopsies, with six of these 11 patients histologically negative. Although relapse-free survival tended to be better in patients with good performance status, in those with poorly differentiated tumors, and in those who received more cisplatin, none of these differences were significant.

A total of 11 patients (39%) have failed; four of these (36% of recurrences) failed locally, six (55%) failed in distant sites (lung, two; bone, two; lung and bone, one; and brain, one), and one (9%) developed a second primary. Median time to failure of these 11 patients was 8 months, but four (36% of the recurrences) failed at 18–29 months. Table 3 shows these results based on the proposed staging system of the Radiation Therapy Oncology Group (RTOG) (18).

Figure 3 shows the Kaplan-Meier survival estimates. Six of the 11 patients have died of their tumor recurrence.

The planned dose of radiation of 60–70 Gy was always delivered, except to two patients who died during treatment and to four who had had prior radiation. An additional patient who had had prior irradiation had the entire radiation field excised when his laryngeal cancer recurred. The subsequent recurrence was then treated to full dosage in this study. A median of 81% of the calculated 5-FU dose and 90% of the cisplatin dose was given.

Toxicity is shown in table 4. Dose-limiting toxic effects were leukopenia, mucositis, and (rarely) severe vomiting. Leukopenia < 2000 cells/mm^3 occurred in 32% of the patients (nadir count, 1100 cells/mm^3). The platelet count nadir was 49,000/mm^3. Confluent mucositis occurred at some point in 32% of the patients. However, in general, the radiation therapists believed that the severity of mucosal and skin toxic effects was less than that expected from irradiation alone, when given by conventional fractionation. Weight loss of > 10% of body weight occurred in 52% of patients due to combined toxicity of cisplatin and radiation. Although the serum creatinine level was never > 2.3 mg/100 ml in any patient during treatment, one patient died of renal failure when treated for an aspiration pneumonia with an aminoglycoside antibiotic 3 weeks after her third cisplatin treatment. Aggravation of aminoglycoside-induced renal failure by cisplatin is well-recognized, so that this event is considered a toxic death. A second patient died suddenly at home, without prodrome, at the end of her fourth rest week and is not considered a toxic death.

A recurrent problem after the vigorous hydration and mannitol diuresis with cisplatin administration was edema of the head and neck structures due to compromise of lymphatic drainage of this region. This responded to furosemide-induced diuresis and dexamethasone, but could be uncomfortable and placed some patients at risk of airway obstruction. As a consequence, we are revising the protocol to omit mannitol, using only furosemide to prevent fluid retention.

Surgery following the combined treatment was complicated by destruction of tissue planes and extensive fibrosis. No severe problems with wound healing occurred in the nine patients who underwent surgery, but one patient developed osteomyelitis of the mandible 1 year later. Late radiation fibrosis (wooden neck) did not occur in the patients who had not had prior radiation. One patient required laryngectomy after 6 months for laryngeal obstruction due to chondritis and edema. This complication occurred after three follow-up biopsies, which we now discourage in the absence of specific indication.

DISCUSSION

The median survival of patients with previously untreated stage IV head and neck cancer without distant spread is 12.7 months based on the RTOG registry study (19).

With the most effective chemotherapy (cisplatin and 5-FU) given prior to surgery and radiation therapy for stage III and IV disease, the median survival is still only 15 months (8).

We have treated a patient population with more advanced disease, including three patients with metastases

TABLE 3.—Recurrence rates and survival of patients based on proposed staging system of the RTOG

Stage	No. of patients	% recurrence	% died	Follow-up (mos)
III	4	0	0	8, 8, 16, 23
IV	14	36	14	15 (6–35)
Other*	10	60	50	21 (7–35)

* 2 had T4 disease (sinus); 3 had M1 disease; and 6 had recurrent disease.

outside the primary and regional nodes and eight previously treated patients, including two who had failed prior therapy with cisplatin/5-FU, five who had recurred after prior radiation therapy, and six who had recurred after surgery. Nevertheless, with a median follow-up of 16 months, our recurrence rate is only 39% and our survival rate is 73%.

The pattern of recurrence among our patients who recurred also differs from that of other studies of advanced head and neck cancer. Fully 55% of recurrences were distant as the only site of failure and 36% were local, whereas normally, > 75% of recurrences would be expected to include regionally treated areas and not more than 20% have distant areas as the only site for recurrence (20,21). This unusual predominance of distant failure may reflect either the unusually advanced disease of our patients or a changing biology of the disease in the face of better regional control.

Besides the impact on survival, the effectiveness of local control has allowed us to attempt to improve the quality of life of these patients, by cautiously limiting surgery to improve function. Conservation of larynx, tongue, and mandible in selected patients has not seemed to alter our results. More experience and longer follow-up is required, but the excellent local control achieved in this patient population with advanced disease has led us, for the first time, to reduce the extent of our surgical resections.

The complete response rate of 57%, using conventional criteria of normalization of involved structures, does not differ substantially from the results of other series, but this result does not reflect the long-term control of disease that has occurred in all of our patients independent

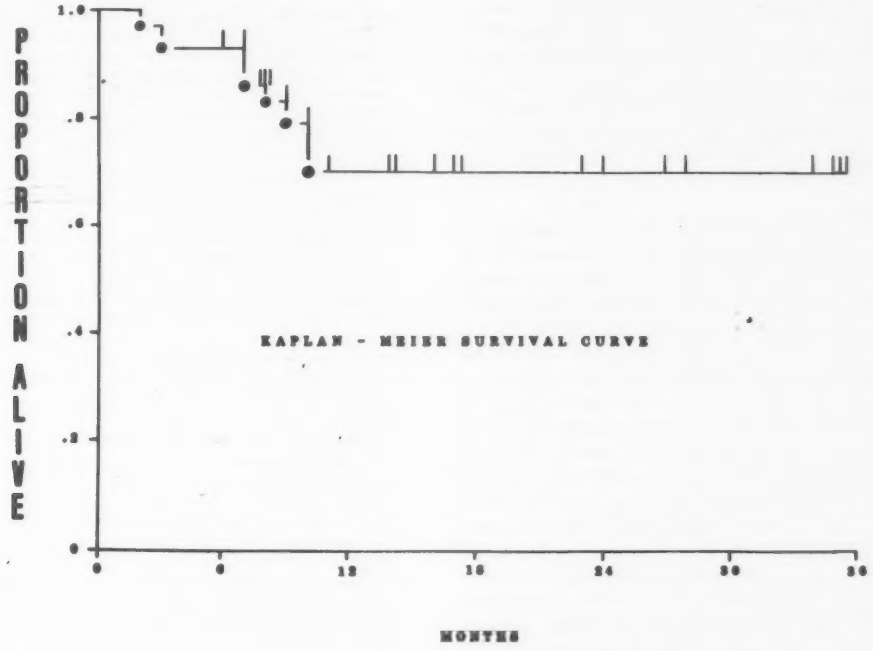

FIGURE 3.—Kaplan-Meier survival estimate of all patients from start of therapy. The first 2 patients who died, died during treatment (*see* text).

TABLE 4.—Toxicity from simultaneous radiation + cisplatin/5-FU in head and neck cancer

Toxic effect	% of patients with toxicity			
	None	Mild	Moderate	Severe
Mucositis	29	18	21	32
Vomiting	21	41	10	24
Weight loss	8	20	20	52
Leukopenia	18	21	29	32
Thrombocytopenia	46	29	21	4
Creatinine evaluation	90	7	0	3*

* Patient developed lethal renal failure during aminoglycoside treatment of an aspiration pneumonia following 3 courses of cisplatin therapy.

of clinical response. Patients with less than complete response have done equally well in follow-up as those achieving complete clinical response. This finding appears to be due to the failure of large masses, and the deformities they produce, to resolve fully despite eradication of viable tumor cells. We were also unable to correlate the rapidity of response with subsequent outcome (data not shown). These observations differ from those of other studies, which emphasize the importance of achieving complete response on subsequent outcome (22). We cannot explain this difference except to note that complete resolution of such advanced disease 6 weeks after completion of therapy may not be a realistic expectation, despite more effective local control.

Because of the exploratory nature of this study, wherein we were interested in toxicity as well as in tumor response, we accepted patients who would ordinarily be treated with chemotherapy only with palliative intent. It is of interest to us that two of the four patients who had had prior radiation therapy, which limited the number of cycles we could readminister, continue in unmaintained disease control for 35 and 14 months, respectively. These responses have encouraged us to selectively treat other such patients.

With such advanced disease in many of the patients entered in this study, we cannot consider our limited follow-up to indicate cure in any patient. We are encouraged, however, by the effectiveness of short-term local control in this advanced disease population, as well as the improved quality of life that this treatment has allowed with conservation of tongue and laryngeal function. The investigators recognize, however, that "encouraging" pilot study results with induction chemotherapy regimens in the past have not led to improved disease control or survival benefit when subjected to randomized, comparative trials (1-4 for example), and that stage IV disease comprises a wide variety of prognostic groupings, making data comparisons outside such clinical trials hazardous. Whether or not combined chemotherapy and radiation therapy with regimens such as the one reported here is superior to sequential treatment, or, indeed, whether chemotherapy has any role in improving long-term disease-free survival of these patients are questions that need to be answered with randomized clinical trials.

REFERENCES

1. WOLF GT, JACOBS C, MAKUCH RW, ET AL. Cisplatin and bleomycin adjuvant chemotherapy in head and neck cancer: results of the head and neck contracts program. *In* Proceedings of the International Conference on Head and Neck Cancer, Baltimore, 1984, p 43.
2. TAYLOR SG, IV, APPLEBAUM E, CALDARELLI D, ET AL. A randomized trial of methotrexate with leucovorin as adjuvant treatment of patients with head and neck squamous cancer. *In* Proceedings of the International Conference on Head and Neck Cancer, Baltimore, 1984, p 43.
3. KUN LE, TOOHILL RJ, HOLOYE PY, ET AL. Effect of induction chemotherapy in advanced cancer of the upper aerodigestive tract: a prospective, randomized trial. *In* Proceedings of the International Conference on Head and Neck Cancer, Baltimore, 1984, p 43.
4. SCHULLER DE, WILSON H, HODGSON S, ET AL. Preoperative reductive chemotherapy for stage III or IV operable epidermoid carcinoma of the oral cavity, oropharynx, hypopharynx or larynx, phase III. A Southwest Oncology Group Study. *In* Proceedings of the International Conference on Head and Neck Cancer, Baltimore, 1984, p 48.
5. CHAN P, BYFIELD J, BARONE R, ET AL. Radiosensitization of human tumors by 5-FU infusions and x-ray therapy: phase I and pharmacokinetic data. Proc Am Assoc Cancer Res and ASCO 20:246, 1979.
6. BYFIELD JE, SHARP TR, FRANKEL SS, ET AL. Phase I and II trial of five-day infused 5-fluorouracil and radiation in advanced cancer of the head and neck. J Clin Oncol 2:406-413, 1984.
7. WEAVER A, FLEMMING S, KISH J, ET AL. *Cis*-platinum and 5-fluorouracil as induction therapy for advanced head and neck cancer. Am J Surg 144:445-448, 1982.
8. AL-SARRAF M, ENSLEY J, KISH J, ET AL. Combined modality therapy with cisplatinum combination in patients with advanced head and neck cancer: results of 5 year experience. *In* Proceedings of the International Conference on Head and Neck Cancer, Baltimore, 1984, p 44.
9. DOUPLE EB, and RICHMOND RC. A review of platinum complex biochemistry suggests a rationale for combined platinum-radiotherapy. Int J Radiat Oncol Biol Phys 5:1335-1339, 1979.
10. OVERGAARD J, and KHAN AR. Selective enhancement of radiation response in a C3H mammary carcinoma by cisplatin. Cancer 65:649-655, 1982.
11. HASELOW RE, ADAMS GS, OKEN MM, ET AL. Simultaneous *cis*-platinum and radiation therapy for locally advanced unresectable head and neck cancer. Proc ASCO 1:201, 1982.
12. HIGI M, HEUSER L, SCHMIDT CH, ET AL. Simultane cisplatin and betrahlungstherapie lokal fortgeschrittener plattenepithelkarzinome der kopf-hals-region. Eine klinische pilotstudie. Dtsch Med Wochenschr 108:1739-1745, 1983.
13. AL-SARRAF M, KINZIE J, MARCIAL V, ET AL. Combination of cisplatinum and radiotherapy in patients with advanced head and neck cancer: Radiation Therapy Oncology Group progress report. Proc ASCO 3:180, 1984.
14. LEIPZIG B, WETMORE SJ, KLUG D, ET AL. *Cis*-platinum sensitization to radiotherapy of squamous cell carcinomas in the head and neck. *In* Proceedings of the International Conference on Head and Neck Can-

cer, Baltimore, 1984, p 42.
15. LO T, WILEY AL, ANSFIELD FJ, ET AL. Combined radiation therapy and 5-fluorouracil for advanced squamous cell carcinoma of the oral cavity and oropharynx: a randomized study. Cancer 126:229–235, 1976.
16. AMERICAN JOINT COMMITTEE FOR CANCER STAGING AND END RESULTS REPORTING. Manual for staging of cancer 1978. Whiting Press, 1978.
17. OKEN MM, CREECH RH, TORMEY DC, ET AL. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649–655, 1982.
18. DAVIS LW, PAJAK TF, MARCIAL V, ET AL. Is the present American Joint Cancer staging (AJC) system for head and neck tumors appropriate for protocol design and results reporting? Proc Am Assoc Cancer Res 25:200, 1984.
19. PAJAK TF, DAVIS LW, FAZEKAS JT, ET AL. Prognostic factors in head and neck tumors for patients initially treated with radiation therapy. Proc ASCO 3:185, 1984.
20. LINDBERG RD. Sites of first failure in head and neck cancer. Cancer Treat Symp 2:21–32, 1983.
21. MARCIAL VA, AMATO DA, and PAJAK TF. Patterns of failure after treatment for cancer of upper respiratory and digestive tracts: a Radiation Therapy Oncology Group report. Cancer Treat Symp 2:33–40, 1983.
22. AL-SARRAF M. Chemotherapy strategies in squamous cell carcinoma of the head and neck. CRC Crit Rev Oncol Hematol 1:323–355, 1984.

Lomustine, Vincristine, and Procarbazine in the Treatment of Metastatic Malignant Melanoma[1]

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Sixty-five previously untreated patients with metastatic malignant melanoma were treated with lomustine, vincristine, and procarbazine. Sixty-four patients were evaluable for response, with a response rate of 13%. Only one complete response was observed, in a patient with nodal disease only. Three partial responses were observed in patients with disease confined to soft tissue, and four partial responses were observed in patients with pulmonary metastases. Median survival for all patients was 22 weeks. We conclude that this regimen offers no improvement compared to other drug combinations. [Cancer Treat Rep 69:941–944, 1985]

The treatment of patients with metastatic malignant melanoma remains disappointingly ineffective. The most widely used single agent, dacarbazine, yields response rates of 17%–22% using various dose schedules (1,2). Other single agents also have activity in this disease, as do various combinations of these agents, but the reported response rates are generally no more encouraging than with dacarbazine alone and the impact on survival has been minimal (3).

In 1980, Carmo-Pereira and co-workers (4) reported an impressive 60% response rate in 30 patients with disseminated malignant melanoma treated with a combination of lomustine, vincristine, and procarbazine. In October 1984, an updated report of this study was published, incorporating the results of an additional 14 patients for a total sample size of 44 (5). The updated response rate was 48%, with complete responses in 11 patients (25%) and partial responses in ten (23%). Responses were seen only in patients with cutaneous, nodal, and/or pulmonary involvement. Green et al (6) used a similar combination of lomustine, vincristine, procarbazine, and cyclophosphamide, and observed two complete responses, two partial responses, and two patients who showed some improvement in disease status out of a total of 12 treated patients (6).

Because of these encouraging results, the National Cancer Institute of Canada Clinical Trials Group initiated a multicenter study using the combination regimen of Carmo-Pereira et al to see if the reported response rates could be duplicated in a larger group of patients.

PATIENTS AND METHODS

Between July 1982 and August 1983, 65 patients were entered in this study. To be eligible, patients had to have measurable metastatic melanoma that had not been previously treated with chemotherapy. No patients were excluded because of poor performance status. One of the 65 patients was inevaluable because

[1]Received Nov 2, 1984; revised Mar 11, 1985; accepted Mar 15, 1985.

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[8]We thank the following investigators who entered patients in the study: Michael P. Thirlwell, MD, Montreal General Hospital; Jean A. Maroun, MD, David J. Stewart, MD, and Daniele J. Perrault, MD, Ontario Cancer Treatment and Research Foundation, Ottawa Clinic; Peter B. McCulloch, MD, Ontario Cancer Treatment and Research Foundation, Hamilton Clinic; David Osoba, MD, Ontario Cancer Treatment and Research Foundation, Toronto-Bayview Clinic; David N. Dalley, MD, Toronto Western Hospital; David P. L. Walde, MB, General and Plummer Memorial Hospital, Sault Ste. Marie; Kenneth S. Wilson, MB, Cancer Control Agency of British Columbia, Victoria Clinic; J. John Berry, MB and L. Martin Jerry, MD, Tom Baker Cancer Centre, Calgary; Leslie Levine, MD and David F. White, MD, Ontario Cancer Treatment and Research Foundation, London Clinic; Andrew M. Arnold, MB, Newfoundland Cancer Treatment and Research Foundation, St. John's; Joseph L. Pater, MD, Ontario Cancer Treatment and Research Foundation, Kingston Clinic; Martin Levitt, MD and Brent A. Schacter, MD, Manitoba Cancer Foundation, Winnipeg.

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he did not return for follow-up after receiving his first treatment. The clinical characteristics of the remaining 64 patients are shown in table 1. Thirty-two patients were female and 32 were male. The median age was 52 years, with a range of 26–78. Fifty-three patients had visceral disease and 11 had disease confined to skin, soft tissue, or lymph nodes. While level of performance status was not an exclusion criterion, information regarding initial performance status was not collected.

The treatment regimen consisted of 150 mg/m^2 of lomustine given orally on Day 1 (maximum dose, 200 mg), 1.4 mg/m^2 of vincristine given iv on Days 1 and 8 (maximum dose, 2 mg), and 100 mg/m^2 of procarbazine given orally on Days 1–10 (maximum daily dose, 150 mg). If there was no myelotoxicity, this regimen was repeated every 28 days. Treatment was delayed until the absolute neutrophil count was $> 2.0 \times 10^9$/L and the platelet count was $> 150 \times 10^9$/L.

Initial investigations included a complete hemogram, serum creatinine, liver function tests, chest x-ray, and any other tests needed to assess extent of metastatic disease. The hemogram was repeated weekly, and all investigations needed to assess response were done before each course of therapy.

Complete response was defined as a complete disappearance of all clinical evidence of disease for a minimum of 4 weeks. Partial response was defined as a $\geq$ 50% decrease in the sum of the products of the perpendicular diameters of bidimensional lesions or a $\geq$ 50% decrease in the linear tumor measurement of unidimensional lesions for a minimum of 4 weeks. Improvement was defined as a 25%–49% decrease in the above parameters of measured lesions for a minimum of 4 weeks. Stable disease was defined as no change, or improvement less than the improvement criteria, or progression less than the progressive disease criteria for at least 8 weeks. Progressive disease was defined as an increase of at least 25% in the size of any lesion or the appearance of a new lesion.

TABLE 1.—Clinical characteristics

Characteristic	No. of patients
Total evaluable	64
Sex	
Male	32
Female	32
Median age in yrs (range): 52 (26–78)	
Primary site	
Eye	10
Head and neck	10
Trunk	25
Extremity	13
Unknown	6
Disease-free interval (yrs)	
< 1	16
1–5	33
> 5	15
Site of metastases	
Skin, soft tissue, or nodal only	11
Visceral	
Liver ± other visceral	16
Lung alone	27
Lung + CNS	3
Lung + bone	5
CNS	1
Intra-abdominal (other than liver)	1

RESULTS

Antitumor Effects

The results are summarized in table 2. One patient had a complete response and seven had partial responses, for an overall response rate of 13%. Five of 32 men (16%) and three of 32 women (9%) responded, while 8% of patients with visceral disease and 36% of patients with only skin, soft tissue, or nodal disease responded. The complete response was seen in a patient with cervical lymph node disease only, and the response lasted 45 weeks. Three of the patients with partial response had disease confined to skin, soft tissue, or lymph node, and the four partial responses seen with visceral disease all involved patients with lung metastases as the only site of visceral involvement. No responses were seen with any other visceral site. The median duration of response for the partial responders was 37 weeks. The median survival for all 64 patients was 22 weeks.

Toxic Effects

Toxic effects with this regimen are summarized in table 3. One patient died after a posttraumatic splenic rupture 17 days after starting treatment. This patient's platelet count at the time of death was 30×10^9/L, but autopsy indicated that the spleen was totally replaced by metastatic melanoma. There were no other episodes of bleeding and no other treatment-related deaths. No episodes of sepsis were reported. Ten patients had neurotoxicity, seven developed peripheral paresthesia, three developed constipation which was felt to be due to paralytic ileus, and one experienced severe jaw pain accompanied by generalized myalgia. Four patients developed a generalized erythematous rash which was felt to be a reaction to procarbazine. The incidence of nausea and vomiting is not reported because it was incompletely documented.

Three patients refused further treatment after the first course because of toxic effects, two because of severe nausea and vomiting and one because of severe jaw pain and generalized myalgia. The patient who died of a ruptured spleen also received only one course. Nineteen patients received only one course, because

TABLE 2.—Patient responses

	No. of patients	No. of patients with—				
		Complete response	Partial response	Improvement	Stable disease	Progressive disease
Total	64	1	7	1	10	45
Male	32	0	5	1	5	21
Female	32	1	2	0	5	24
Skin, soft tissue, or lymph nodes only	11	1	3	1	1	5
Visceral						
Liver ± other visceral	16	0	0	0	1	15
Lung alone	27	0	4	0	7	16
Lung + CNS	3	0	0	0	1	2
Lung + bone	5	0	0	0	0	5
CNS	1	0	0	0	0	1
Intra-abdominal (other than liver)	1	0	0	0	0	1

definite progressive disease developed before the second course could be initiated. The remaining 41 patients received two or more courses of treatment.

DISCUSSION

The response rate observed in our sample population of 64 patients with metastatic melanoma was 13% with this regimen of lomustine, vincristine, and procarbazine. The 95% confidence limits for this sample size and response rate are 6%–23%. This is far below the response rate observed by Carmo-Pereira et al, and is most likely due to a difference in prognostic characteristics between the two patient populations. The most obvious difference is the site of disease. In Carmo-Pereira's study (5), 28 of the 44 patients (64%) had disease confined to skin and/or lymph nodes, and 16 of the 21 responses occurred in this group. In our study, there was also a higher response rate seen in the soft tissue sites of disease, but only 11 of 64 patients (17%) in our series had no visceral involvement. Four of our eight responses were seen in this group. The remaining four responses were seen in patients with lung metastases as the only site of visceral involvement, but the total number of patients in this category was 27, giving a relatively low response rate of 15% (four of 27) in patients with visceral metastases confined to the lung. Like Carmo-Pereira et al, we found the regimen to be completely ineffective in the 26 patients whose disease had spread to visceral sites other than the lung. Therefore, we conclude that, while this regimen appears to be more effective when metastases are limited to soft tissue as compared to visceral spread, it is no more effective than other previously reported regimens.

TABLE 3.—Toxic effects

Toxic effect	No. of patients (%)
Leukopenia (nadir cell count × 10^9/L)	
1.0–2.0	8 (13)
< 1.0	1 (2)
Thrombocytopenia (nadir cell count × 10^9/L)	
50–100	15 (23)
25–50	8 (13)
< 25	2 (3)
Neurotoxicity	10 (16)
Skin rash	4 (6)

REFERENCES

1. COMIS RL. DTIC (NSC-45388) in malignant melanoma: a perspective. Cancer Treat Rep 60:165–176, 1976.
2. PRITCHARD KI, QUIRT IC, COWAN DH, ET AL. DTIC therapy in metastatic malignant melanoma: a simplified dose schedule. Cancer Treat Rep 64:1123–1126, 1980.
3. BELLST RE, MASTRANGELO MJ, BARD D, ET AL. Chemotherapy of metastatic melanoma. *In* Human Malignant Melanoma (Clark WH, Jr, Goldman LI, and Mastrangelo MJ, eds). New York, Grune and Stratton, 1979, pp 325–354.
4. CARMO-PEREIRA J, COSTA FO, PIMENTEL P, ET AL. Combination cytotoxic chemotherapy with CCNU, procarbazine, and vincristine in disseminated cutaneous malignant melanoma: 3 years' followup. Cancer Treat Rep 64:143–145, 1980.
5. CARMO-PEREIRA J, COSTA FO, and HENRIQUES E. Combination cytotoxic chemotherapy with procarbazine, vincristine, and lomus-

tine (POC) in disseminated malignant melanoma: 8 years' follow-up. Cancer Treat Rep 68:1211-1214, 1984.
6. GREEN MR, DILLMAN RO, and HORTON C. Procarbazine, vincristine, CCNU, and cyclophosphamide (POCC) in the treatment of metastatic malignant melanoma. Cancer Treat Rep 64:139-142, 1980.

Comparison of Vindesine Plus Cisplatin or Vindesine Plus Mitomycin in the Treatment of Advanced Non-Small Cell Lung Cancer[1]

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Fifty-eight patients with advanced non-small cell lung cancer were randomly allocated to receive vindesine (3 mg/m^2 every week) plus either cisplatin (80 mg/m^2 every 3 weeks) or mitomycin (8 mg/m^2 weekly × 3, then every 3 weeks). No patients achieved complete response. Among the 28 patients treated with vindesine plus cisplatin, there were 12 partial responders (42.9%); among the 30 patients treated with vindesine plus mitomycin, there were only three partial responders (10%) ($P < 0.005$). The median duration of response was 11.5 weeks (range, 4–25) in the patients treated with vindesine plus cisplatin. The median survival times for patients treated with vindesine plus cisplatin and vindesine plus mitomycin were 10.1 and 10.2 months, respectively; there was no statistical difference in survival time between the two groups. Initial performance status was the strong predictor of patient survival. Toxic effects, including moderate myelosuppression, nephrotoxicity, peripheral neuropathy, and gastrointestinal symptoms, were generally manageable. The combination of vindesine and cisplatin appears to be effective against advanced non-small cell lung cancer. [Cancer Treat Rep 69:945–951, 1985]

Lung cancer is the second cause of death from cancer in Japan, with 25,000 deaths estimated in 1983 (1). The incidence of lung cancer has recently increased, and it is thought that lung cancer may surpass stomach cancer within 10 years in Japan. The prognosis for lung cancer patients remains poor, with a 5-year survival rate of < 10%. Non-small cell lung cancer (NSCLC) accounts for > 85% of the cases of lung cancer, and curative operations have been performed on < 20% of the patients (2); therefore, > 80% of the patients with NSCLC have advanced-stage lung cancer which is inoperable at presentation. In addition, the 5-year survival rate for patients who had curative resections is about 50%. From these points of view, the need for effective systemic chemotherapy is clear. Despite recent advances in chemotherapy, the treatment of inoperable NSCLC remains poor. Recently, Gralla et al (3) reported a response rate of 43% in patients with NSCLC who were treated with the combination of cisplatin-vindesine, and they also observed that a high dose of cisplatin resulted in longer median survival of responding patients than a lower dose (21.7 vs 10 months). In their review, Bakowski and Crouch (4) stated that the effective chemotherapeutic agents used as single agents against NSCLC are cisplatin (5-7), ifosfamide (8-10), vindesine (11-15), and mitomycin (16-18). In the National Cancer Center Hospital (Tokyo, Japan), cisplatin, vindesine, and mitomycin, as single chemotherapeutic agents, have been reported to produce response rates of 17.6% (19), 17.6% (20), and 18.9% (21), respectively, for patients with NSCLC. Combination chemotherapy has now begun to yield results clearly superior to those achieved with single agents (4). We therefore initiated a randomized clinical trial of vindesine plus either cisplatin or mitomycin in advanced NSCLC to evaluate the two treatment regimens.

MATERIALS AND METHODS

From July 1982 to April 1984, patients with histologically proven advanced NSCLC were eligible if they had an expected survival of > 6 weeks, measurable lesions, Eastern Cooperative Oncology Group (ECOG) performance score ≤ 3, wbc count ≥ 4000/mm^3, platelet count ≥ 100,000/mm^3, total serum bilirubin < 3 mg/dl, SGOT and SGPT less than twice the normal range, and creatinine clearance of at least 60 ml/minute, and if

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they had given written informed consent. None of the patients had had prior chemotherapy, radiotherapy, or surgery. Pretreatment evaluation included medical history, physical examination, cbc, urinary creatinine clearance, relevant laboratory tests, chest roentgenogram, ECG, and urinalysis. All patients had radionuclide bone scan, computerized tomography of brain and thorax, and ultrasonography or computerized tomography of abdomen. Physical examinations, cbcs, and chest roentgenograms were obtained weekly for the first 6 weeks of treatment and every 2-4 weeks thereafter. Biochemical tests and serum electrolytes were repeated at 2-week intervals, and creatinine clearance was done before each course of cisplatin. The disease was defined as limited if the tumor was confined by all clinical measurements to one hemithorax, including mediastinal or ipsilateral supraclavicular nodes. Disease beyond these confines was classified as extensive.

Patients were randomly assigned to receive vindesine plus either cisplatin or mitomycin. Vindesine (3 mg/m^2) was given in both treatment regimens by rapid iv injection once a week for the first 5 weeks and then on a schedule of every 2 weeks. Cisplatin (80 mg/m^2) was given iv over 20 minutes with prehydration and mannitol-induced diuresis on Days 1, 22, and 43, and every 6 weeks thereafter. Mitomycin (8 mg/m^2) was given by rapid iv infusion once a week for the first 3 weeks and then every 3 weeks. Subsequently, a full dose of the drug was given if the leukocyte count was $> 3000/mm^3$ and the platelet count was $> 75,000/mm^3$, and vindesine or mitomycin was withheld until recovery of the count if the leukocyte or platelet count fell below this level. Chemotherapy was continued until there was evidence of progressive disease or unacceptable toxicity. Radiation could be given to treat documented brain metastasis and symptomatic bone lesions. CNS metastasis was present at the time of diagnosis in two (7%) patients allocated to receive vindesine plus cisplatin and in two (7%) allocated to receive vindesine plus mitomycin; they received irradiation (4000-5000 rads in 20-25 fractions for 4-5 weeks) to the brain immediately prior to the initiation of chemotherapy. In this study, no prophylactic cranial irradiation was performed. Clearly definable pulmonary tumors and lymph nodes or subcutaneous mass were accepted as measurable disease. Pleural effusion, hepatomegaly, and abnormalities detected only on radionuclide scans were not accepted as criteria for evaluation, but were followed. Computerized tomography and ultrasonography of the liver or abdomen clearly revealing measurable lesions were acceptable.

Complete response (CR) was defined as the complete disappearance of all known disease as indicated by examinations at least 4 weeks apart. Partial response (PR) was defined as a > 50% reduction in the product of the longest diameter and widest perpendicular diameter of all measurable lesions for ≥ 4 weeks without the appearance of new lesions or growth of other measurable lesions. Minor response was defined as a 25%-50% reduction in tumor size without the appearance of new lesions or growth of other measurable lesions lasting ≥ 4 weeks. Stable disease was defined as a lack of tumor progression for ≥ 4 weeks. Tumor progression was defined as a $\geq 25\%$ increase in tumor area or the appearance of new lesions. The duration of response was defined as the time from onset of > 50% regression of the tumor to the first sign of progressive disease. Survival curves from Day 1 of treatment until death were calculated by the method of Kaplan and Meier (22), and tests of difference in survival distribution were based on a generalized Wilcoxon-Gehan test (23). The chi-square test was used to test for differences in response and toxicity between the two treatment regimens. Other results with the two treatment arms were compared by using an unpaired t test.

RESULTS

The 58 patients entered in this study were all evaluable for therapeutic response and toxicity; their characteristics are summarized in table 1. The two treatment groups were well-matched for sex ratio, performance status, extent of disease, weight loss before treatment, and histologic type of lung cancer, but not for age ($P < 0.02$).

Response Data

No patients achieved CR to either treatment regimen. Among the 28 patients treated with cisplatin plus vindesine, there were 12 partial responders (42.9%) with adenocarcinoma (six), squamous cell carcinoma (three), and large cell carcinoma (three), and five minor responders with adenocarcinoma (three) and squamous cell carcinoma (two). Among the 30 patients treated with vindesine plus mitomycin, there were three partial responders (10%) with adenocarcinoma, and five minor responders with adenocarcinoma (table 1). Cisplatin plus vindesine was more active against NSCLC than mitomycin plus vindesine ($P < 0.005$). Tumor regression of > 50% in the 12 patients who responded to vindesine plus cisplatin and in the three who responded to vindesine plus mitomycin was observed at 5 weeks (range, 2.6-11.3) and 6 weeks (range, 4-7), respectively, after the beginning of therapy. Three of the 12 patients who achieved PR with cisplatin plus vindesine required three doses of cisplatin up to tumor regression of > 50%. The observed responses and their distribution according to selected prognostic factors are shown in table 1. Patients who progressed on vindesine plus

TABLE 1.—Patient characteristics, response, and survival*

Characteristic	Vindesine + cisplatin	Vindesine + mitomycin	Median survival time (mos)
Total	28 (12)	30 (3)	
Sex			
Male	18 (7)	20	10.3
Female	10 (5)	10 (3)	10.2
Median age in yrs (range)	65 (42-73)	55 (31-72)	
ECOG performance status			
0	3 (1)	5 (1)	14.9
1	15 (7)	18	10.3
2	8 (3)	6 (2)	6.1
3	2 (1)	1	4.5
Extent of disease			
Limited	12 (4)	11 (1)	14.5
Extensive	16 (8)	19 (2)	6.7
Weight loss (≥ 10% body weight)			
Yes	6 (2)	8 (1)	6.3
No	16 (8)	21 (2)	10.2
Unclear	6 (2)	1	
Histology			
Adenocarcinoma	18 (6)	21 (3)	10.8
Squamous cell carcinoma	5 (3)	5	8.5
Large cell carcinoma	4 (3)	4	7.6
Adenosquamous cell carcinoma	1		
Response			
CR	0	0	—
PR	12	3	10.3
Minor response	5	5	11.7
Stable disease	8	16	11.0
Progressive disease	3	6	3.8

*Unless specified otherwise, values = No. of patients (No. of responses).

mitomycin and met certain eligibility criteria to receive further chemotherapy received cisplatin (80 mg/m^2 every 3 weeks) as a single chemotherapeutic agent, and two of 24 patients achieved PR lasting 10 and 15 weeks. Seven patients (25%) treated with vindesine plus cisplatin and five (17%) treated with vindesine plus mitomycin who had limited disease that could be encompassed within one radiation therapy port received radiotherapy (5000-6000 rads/25-30 fractions/5-6 weeks) for local progression after chemotherapy.

The median duration of response was 11.5 weeks (range, 4-25) in the patients treated with vindesine plus cisplatin, and there were three PR lasting 4, 14, and 15 weeks in the patients treated with vindesine plus mitomycin. Three of the 12 responding patients had vindesine plus cisplatin treatment discontinued in remission: one patient underwent an operation of lung tumor at 4 weeks after tumor regression; one could not receive the second cisplatin dose because of a fall in creatinine clearance of < 40 ml/minute; and one refused further cisplatin after the second dose because of severe cisplatin-induced emesis.

The median survival times for the 28 patients treated with vindesine plus cisplatin and for the 30 patients treated with vindesine plus mitomycin were 10.1 and 10.2 months, respectively. At the final analysis in December 1984, there were ten and six survivors in the vindesine plus cisplatin and vindesine plus mitomycin groups, respectively, and there is no difference in survival time between the two groups (fig 1). Partial responders survived a median time of 10.2 months (range, 4.5-28.3), with three of 15 patients still alive; this was significantly superior to the median of 3.8 months (range, 2.0-7.9) for patients who had disease progression ($P < 0.01$), but compared to the median of 11.0 months (range, 2.8-25.4+) for patients with minor response or stable disease, no significant difference was observed. Thirteen of 34 patients with minor response or stable disease are still alive. With regard to initial performance status (PS), the numbers of ambulatory (PS of 0 or 1) patients who had PR, minor response or stable disease, and disease progression were eight (60%), 26 (79%), and five (55%), respectively, although these numbers were not statistically significant.

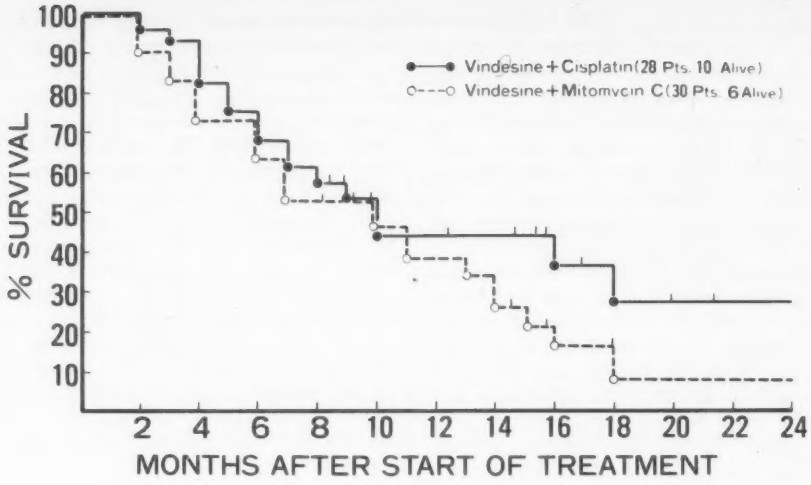

FIGURE 1.—Survival time from the start of chemotherapy for all patients with NSCLC treated with vindesine and either cisplatin or mitomycin. Tick mark (⊥) indicates last follow-up.

The median survival time was 11.6 months for ambulatory patients and 6.0 months for nonambulatory patients (PS of 2 or 3); the difference was statistically significant ($P < 0.01$), although response rates did not vary according to PS. As indicated in table 1, prior weight loss and extent of disease were significantly associated with survival ($P < 0.01$ for both). There was no difference in survival for men versus women or for patients with adenocarcinoma versus squamous cell carcinoma. In patients with extensive disease, the median survival for ten patients achieving PR was 10.3 months, as compared to a median survival of 3.8 months for those with disease progression ($P = 0.013$), but compared to the median of 9.0 months for 16 patients with minor response or stable disease, no significant difference was demonstrated. A statistically significant difference in survival was not achieved between 22 ambulatory patients and 13 nonambulatory patients (10.3 vs 6.0 months, respectively; $P = 0.085$) within the category of extensive disease, possibly because of the small number of patients. Nine of the 12 patients who achieved PR with vindesine plus cisplatin and all three who achieved PR with vindesine plus mitomycin had died by the time of this report. Of these responders, three of the nine patients who received vindesine plus cisplatin and two of the three who received vindesine plus mitomycin (including one with brain metastasis prior to chemotherapy from each of the above two groups) died from brain metastases, although four of the five patients had received cranial irradiation when brain metastases were documented, and, in three patients (two treated with cisplatin plus vindesine and one treated with mitomycin plus vindesine), there was no evidence of other clinical progression except brain.

Toxicity Data

The hematologic toxicity is summarized in table 2.

TABLE 2.—Hematologic toxicity*

	Vindesine + cisplatin	Vindesine + mitomycin
Wbc count nadir (cells/mm³)		
Median	1650	1700
Range	800-3800	800-3200
≥ 4000	0 (0)	0 (0)
3000-3900	1 (3)	1 (3)
2000-2900	7 (25)	8 (27)
1000-1900	17 (61)	20 (67)
< 1000	3 (11)	1 (3)
Platelet count nadir (cells/mm³)		
Median	128,000	77,000
Range	47,000-248,000	8000-205,000
≥ 100,000	19 (68)	11 (37)
75,000-99,000	5 (18)	4 (13)
50,000-74,000	3 (11)	9 (30)
< 50,000	1 (3)	6 (20)
Hemoglobin (g/dl)		
Median	8.8	10.5
Range	7.5-12.0	7.8-13.2
≥ 11	1 (3)	8 (27)
9.5-10.9	6 (21)	14 (46)
8.0-9.4	18 (64)	7 (23)
< 8.0	3 (11)	1 (3)

* Unless specified otherwise, values = No. of patients (%).

Leukopenia was the common manifestation of marrow toxicity in both treatment regimens, and it was the major hematologic dose-limiting toxic effect. Ninety-seven percent of the patients treated with vindesine plus cisplatin and vindesine plus mitomycin developed leukopenia (< 3000/mm^3), with a median count nadir of 1650/mm^3 on Day 20 (range, Day 10-46) in patients treated with vindesine plus cisplatin, and 1700/mm^3 on Day 14.5 (range, Day 10-17) in patients treated with vindesine plus mitomycin. Thrombocytopenia (< 75,000 cells/mm^3) occurred more frequently in patients treated with mitomycin plus vindesine (50%), with a median count nadir of 77,000 cells/mm^3 on Day 34.5 (range, Day 6-63), than in the cisplatin plus vindesine arm. Anemia was more pronounced in patients treated with cisplatin plus vindesine, with a median hemoglobin count nadir of 8.8 g/dl, than in patients treated with mitomycin plus vindesine, with a median nadir of 10.5 g/dl ($P < 0.005$), without evidence of gastrointestinal bleeding. No life-threatening infections or bleeding episodes were observed as a result of bone marrow suppression in either treatment arm.

Nonhematologic toxic effects are presented in table 3. Nearly all patients treated with cisplatin plus vindesine experienced nausea and vomiting, but cisplatin-induced emesis was markedly reduced by the concomitant use of iv high-dose metoclopramide (2 mg/kg iv every 2 hours for four doses). Alopecia and mild to moderate peripheral neuropathy were common in both treatment groups. Reversible abnormal liver function was observed in about one-third of the patients treated with mitomycin plus vindesine. Loss of hearing was clinically significant in two patients treated with cisplatin plus vindesine, although routine audiometric studies were not performed. Peak serum creatinine levels > 1.5 mg/dl were found in 13 (47%) of the 28 patients treated with cisplatin plus vindesine, and the levels returned to < 1.5 mg/dl within 5 weeks in ten of the 13 patients. No patients developed severe renal insufficiency.

In patients treated with vindesine plus cisplatin, the median total doses of vindesine and cisplatin per patient were 18 mg/m^2 (range, 9-60) and 160 mg/m^2 (range, 80-560), respectively. In patients treated with vindesine plus mitomycin, the median total doses of vindesine and mitomycin per patient were 12 mg/m^2 (range, 9-21) and 24 mg/m^2 (range, 24-56), respectively.

TABLE 3.—Nonhematologic toxicity

	No. of patients (%)	
	Vindesine + cisplatin (N = 28)	Vindesine + mitomycin (N = 30)
Nausea or vomiting	26 (93)	9 (30)
Diarrhea	9 (32)	8 (27)
Constipation	5 (18)	1 (3)
Stomatitis	4 (14)	4 (13)
Fever	5 (18)	5 (17)
Alopecia	17 (61)	15 (50)
Eruption or pigmentation	9 (32)	6 (20)
Abnormal liver function	4 (14)	9 (30)
Ototoxicity	2 (7)	1 (3)
Peripheral neurotoxicity	9 (32)	15 (50)
Maximum serum creatinine (mg/dl)		
≤ 1.4	15 (53)	29 (97)
1.5-2.0	8 (29)	1 (3)
2.1-3.4	3 (11)	0
≥ 3.5	2 (7)	0

DISCUSSION

A variety of combination regimens have been introduced for the treatment of NSCLC. The response rate in pilot studies may have been encouraging, but follow-up and randomized trials have usually been disappointing and have failed to demonstrate a meaningful impact on survival; even among responding patients, the median survival is usually not > 1 year. Recently, the combination of vindesine and cisplatin has shown marked activity against NSCLC, producing a major objective response in 43% of previously untreated patients (3), and the effectiveness of this combination has been confirmed at several institutions (24-26). In addition, in human tumor clonogenic assays, in vitro results showed synergistic or greater than expected cell killing by the combination of cisplatin and vindesine in ten of 17 patients with carcinomas of the lung (27). The 43% response rate for the 28 patients in this study, the first randomly allocated study to compare vindesine plus cisplatin and vindesine plus mitomycin in NSCLC, supports previous reports (3,24-26) of the effectiveness of the combination of vinca alkaloids and cisplatin in the treatment of NSCLC (3,24-26,30-32).

On the other hand, the response rate for the combination of vindesine and mitomycin was only 10%. Luedke et al (28) reported that the rate of response of NSCLC to the combination of vindesine and mitomycin was 35% (21 responses among 59 patients). Kris et al (29) also reported a 33% response (18 responses among 55 patients) to the combination of vindesine and mitomycin in previously untreated patients with NSCLC, and stated that the combination of vindesine and mitomycin appeared to be more effective than vindesine alone against NSCLC. The reason for the lack of an antitumor effect of vindesine plus mitomycin against NSCLC in this study in contrast with the results presented in other publications (28,29) remains unclear; however, one might conclude that the lack of an antitumor effect in this trial may have been caused by the different schedule of mitomycin treatment, and the fre-

quent attenuation of vindesine was necessitated by mitomycin (weekly dose of 8 mg/m^2)-induced myelotoxicity in this trial.

Despite the fact that vindesine plus cisplatin showed increased activity against NSCLC, there was no difference in survival rates between patients treated with vindesine plus cisplatin (10.1 months) and those treated with vindesine plus mitomycin (10.2 months). In addition, the median survival time of responding patients treated with vindesine plus cisplatin was not > 1 year. The treatment program of this study consisted of 80 mg/m^2 of cisplatin given iv 3 weeks apart. This dose was chosen since it was the highest dose we felt could be given safely in an outpatient clinic. As stated previously, Gralla et al (3) reported that high-dose cisplatin (120 mg/m^2) was superior to low-dose cisplatin (60 mg/m^2) in regard to the median duration of response and survival (21.7 vs 10 months) for responding patients. Recently, high-dose cisplatin (200 mg/m^2), as part of an intensive polydrug regimen, has been administered without any increase in nephrotoxicity by using vigorous saline hydration and 3% saline as the vehicle for drug delivery. This treatment has resulted in a marked increase in the response rate of patients with testicular cancer, and three responses were obtained in six patients with ovarian cancer for whom standard-dose cisplatin therapy had failed (33). Therefore, we are now testing high-dose cisplatin against NSCLC.

REFERENCES

1. STATISTICS AND INFORMATION DEPARTMENT, MINISTER'S SECRETARIAT, MINISTRY OF HEALTH AND WELFARE. Vital Statistics, Japan, 1983.
2. YOSHIMURA K. Treatments and diagnosis of lung cancer—field study results: report from Japan Lung Cancer TNM Classification Committee. Jpn J Cancer Chemother 10:708-714, 1983.
3. GRALLA RJ, CASPER ES, KELSEN DP, ET AL. Cisplatin and vindesine combination chemotherapy for advanced carcinoma of the lung: a randomized trial investigating two dosage schedules. Ann Intern Med 95:414-420, 1981.
4. BAKOWSKI MT, and CROUCH JC. Chemotherapy of non-small cell lung cancer: a reappraisal and a look to the future. Cancer Treat Rev 10:159-172, 1983.
5. CASPER ES, GRALLA RJ, KELSEN DP, ET AL. Phase II study of high-dose *cis*-dichlorodiammineplatinum(II) in the treatment of non-small cell lung cancer. Cancer Treat Rep 63:2107-2109, 1979.
6. DE JAGER R, LONGEVAL E, and KLASTERSKY J. High-dose cisplatin with fluid and mannitol-induced diuresis in advanced lung cancer: a phase II clinical trial of the EORTC lung cancer working party (Belgium). Cancer Treat Rep 64:1341-1346, 1980.
7. VOGL SE, BERENZWEIG M, CAMACHO F, ET AL. Efficacy study of intensive *cis*-platin therapy in advanced non-small cell bronchogenic carcinoma. Cancer 50:24-26, 1982.
8. MORGAN LR, POSEY LE, RAINEY J, ET AL. Ifosfamide: a weekly dose fractionated schedule in bronchogenic carcinoma. Cancer Treat Rep 65:693-695, 1981.
9. HARRISON EF, HAWKE JE, COSTANZI JJ, ET AL. Single-dose ifosfamide: efficacy studies in non-small cell lung cancer. Semin Oncol 9:56-60, 1982.
10. COSTANZI JJ, MORGAN LR, and HOKANSON J. Ifosfamide in the treatment of extensive non-oat cell carcinoma of the lung. Semin Oncol 9:61-65, 1982.
11. GRALLA RJ, TAN CTC, and YOUNG CW. Vindesine: a review of phase II trials. Cancer Chemother Pharmacol 2:271-274, 1979.
12. MATTSON K, HOLSTI LR, SALMO M, ET AL. Vindesine in the treatment of small cell and non-small cell bronchogenic carcinoma: preliminary results. Cancer Treat Rev 7:65-70, 1980.
13. FURNAS BE, WILLIAMS SD, EINHORN LH, ET AL. Vindesine: an effective agent in the treatment of non-small cell lung cancer. Cancer Treat Rep 66:1709-1711, 1982.
14. ØSTERLIND K, HØRBOV S, DOMBERNOWSKY P, ET AL. Vindesine in the treatment of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma of the lung. Cancer Treat Rep 66:305-309, 1982.
15. LUEDKE SL, LUEDKE DW, PETRUSKA P, ET AL. Vindesine (VDS) monochemotherapy for non-small cell lung cancer: a report of 45 cases. Cancer Treat Rep 66:1409-1411, 1982.
16. SAMSON MK, COMIS RL, BAKER LH, ET AL. Mitomycin C in advanced adenocarcinoma and large cell carcinoma of the lung. Cancer Treat Rep 62:163-165, 1978.
17. KOONS LS, HARRIS DT, and ENGSTROM PF. Mitomycin C chemotherapy in advanced squamous cell cancer of the lung. Proc Am Assoc Cancer Res and ASCO 19:326, 1978.
18. RUCKDESCHEL JC, MEHTA CR, SALAZAR OM, ET AL. Chemotherapy for inoperable, non-small cell bronchogenic carcinoma: EST 2575, Generation II. Cancer Treat Rep 65:965-972, 1981.
19. TOMINAGA K, SAIJO N, EGUCHI K, ET AL. Preliminary phase II study of *cis*-dichlorodiammineplatinum (II) in patients with non-small cell carcinoma of the lung and metastatic pulmonary tumor. Jpn J Cancer Clin 30:1344-1350, 1984.
20. SHIBUYA M, SAIJO N, SHIMIZU H, ET AL. Phase II study of vindesine in patients with carcinoma of the lung and metastatic pulmonary tumor. Jpn J Cancer Chemother 9:1808-1813, 1982.
21. SAIJO N, NIITANI H, TOMINAGA K, ET AL. Comparison of survival in non-resected well and poorly differentiated adenocarcinoma of the lung. J Cancer Res Clin Oncol 97:71-79, 1980.
22. KAPLAN EL, and MEIER P. Non-parametric estimation from incomplete observations. J Am Stat Assoc 63:457-481, 1958.
23. GEHAN E. A generalized Wilcoxon test for comparing arbitrarily single-censored samples. Biometrika 52:203-224, 1965.
24. ELLIOT JA, AHMEDZAI S, STEVENSON RD, ET AL. Randomized comparison of vindesine (VDS) versus vindesine plus cis-platinum (DDP) in inoperable non-small cell lung cancer (NSCLC). *In* Proceedings of the Third World Conference on Lung Cancer, Tokyo, Japan, May 17-20, 1982.
25. GRÖHEN P, NIITAMO S, MATTSON K, ET AL. Combination chemotherapy in advanced non-small cell lung cancer. *In* Proceedings of the Third World Conference on Lung Cancer, Tokyo, Japan, May 17-20, 1982.
26. DHINGRA HM, MURPHY WK, CHIVTEN DF, ET AL. Chemotherapy with cisplatin (DDP) with vindesine (VDS) and/or VP16 for advanced adeno-squamous cell lung cancer (ASCLC) and its effect on P.S. (ECOG) of patients. Proc ASCO 3:220, 1984.
27. KANZAWA F, MATSUSHIMA Y, HOSHI A, ET AL. The use of human tumor clonogenic assay in predicting combination effect of cisplatin plus vindesine in carcinoma of the lung. Gann, 1984. In press.
28. LUEDKE D, LUEDKE S, MARTELLO O, ET AL. Response of non-small cell lung cancer (NSCLC) to vindesine (VDS) and mitomycin C (MITO): a Southeastern Cancer Study Group pilot study. Proc ASCO 2:190, 1983.
29. KRIS MG, GRALLA RJ, KELSEN DP, ET AL. Trials adding mitomycin to vindesine and vindesine plus cisplatin in non-small cell lung cancer (NSCLC). Proc ASCO 3:225, 1984.
30. KALMAN LA, KRIS MG, GRALLA RJ, ET AL. Vinca alkaloid and cisplatin combination therapy in non-small cell lung cancer (NSCLC). Proc ASCO 2:201, 1983.
31. HALIBEY B, LIPPMAN A, CUSTODIO M, ET AL. Clinical trials of *cis*-

diamminedichloroplatinum (CDDP) and vinblastine (VLB) in non-small cell lung carcinoma. Proc ASCO 2:202, 1983.
32. ESSEESSE I, BABOTT D, ROTHENBERG SP, ET AL. *Cis*-diammine-dichloroplatinum (II) (CP) and vinblastine (VLB) treatment of advanced non-small cell lung cancer (NSCLC). Proc ASCO 3:216, 1984.
33. OZOLS RF, CORDEN BJ, JACOB J, ET AL. High-dose cisplatin in hypertonic saline. Ann Intern Med 100:19–24, 1984.

Autologous Bone Marrow Transplantation With Marrow Decontaminated by Immunotoxin T 101 in the Treatment of Leukemia and Lymphoma: First Clinical Observations[1,2,3]

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Four patients with T-cell malignancies of poor prognosis (three with non-Hodgkin's lymphoma and one with acute lymphoblastic leukemia) received the following consolidation therapy for complete or partial remission: cyclophosphamide (120 mg/kg) plus total-body irradiation, followed by reinfusion of cryopreserved autologous marrow previously purged in vitro by immunotoxin T 101 (SR 41322). This immunotoxin is made of the murine monoclonal T 101 antibody coupled to chain A of ricin. The doses of immunotoxin used were 10^{-9} and 10^{-8} M, and the durations of incubation were 4 and 20 hours at 37°C. Recovery of progenitors CFUc and BFUe was total following incubation with immunotoxin T 101, but diminished after cryopreservation (15%–80% for CFUc, 33%–47% for BFUe), suggesting an increased fragility of the incubated progenitors to freezing. In every case, hematopoietic recovery occurred within normal time periods, with a wbc count > 10^9/L and a platelet count > 50 × 10^9/L on Day 22 (range, 15–31) and Day 21 (range, 22–47), respectively, demonstrating the feasibility of autologous bone marrow transplantation with marrow pretreated by immunotoxin. However, the slow recovery of lymphocytes and the development of severe infections in two patients may indicate that an in-depth study of immunological reconstitution after in vitro treatment of the marrow with immunotoxin T 101 is necessary. [Cancer Treat Rep 69:953–959, 1985]

Autologous bone marrow transplantation (ABMT) is currently used by many groups for treating acute leukemia (1,2) and non-Hodgkin's lymphoma (3–6). However, the bone marrow of patients with leukemia collected during complete remission may contain residual tumor cells; this may also be encountered at times, depending on the type of pathology, in non-Hodgkin's malignant lymphoma (7,8). A number of products are currently being tested for purging the bone marrow of contaminating cells (9,10).

Immunotoxin T 101 (SR 41322) is a hybrid molecule made of the murine monoclonal antibody T 101 bound by a disulfide bridge to the A chain of the ricin molecule. The T 101 monoclonal antibody binds the T 65 antigen, which is present on the surface of normal and malignant T cells but absent on neutrophil and erythroblast progenitors (11). Following the fixation of the T 101 antibody on the target cell, the A chain of ricin penetrates the cytoplasm, which leads to cell destruction through protein synthesis inhibition.

Here we report our first clinical experience using immunotoxin T 101 with specific anti-T action in the framework of a phase I trial in four patients with T-cell acute lymphoblastic leukemia or T-cell lymphoma. In these cases, the normal course of the autograft, after total-body irradiation, confirmed the absence of immunotoxin

[1]Received Nov 19, 1984; revised Mar 26, 1985; accepted Mar 28, 1985.

[2]Supported in part by funds from INSERM (No. 1290022) and ARC (No. 6113).

[3]Presented in part at the 1984 meeting of the French National Society for Blood Transfusion, Brest, France, May 7, 1984.

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[9]We thank Mrs. M. C. Leguen and Mr. C. Baillou for their expert technical assistance, and Miss M. Veillard for record keeping and preparation of the manuscript.

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toxicity for the erythroblast, neutrophil, and platelet precursors, already observed in vitro (12). On the other hand, assessment of the immunological repercussion of this specifically anti-T immunotoxin and its antitumor efficacy calls for a more prolonged study.

MATERIALS AND METHODS

Patients

Patients chosen for inclusion in the study had the following: (*a*) a tumor of lymphoblastic or T-cell origin, proven immunologically by a study of the membrane antigens with a battery of fluorescent monoclonal antibodies; and (*b*) clinical manifestations of unfavorable prognosis.

Three patients (Nos. 1-3) had lymphoblastic T-cell lymphoma initially in stage III or IV, with a mediastinal mass larger than a third of the thoracic cage and pleuropericardial effusion. Initial bone marrow involvement was demonstrated in Patient 2. Total-body irradiation and autografting were done in two patients (Nos. 1 and 3) in consolidation mode during the first complete remission, and in Patient 3 at the time of subdiaphragmatic relapse. Patient 4 had acute T-cell lymphoblastic leukemia in second remission after systemic and meningeal relapse. Clinical details are given in table 1.

Bone Marrow Collection and In Vitro Studies

The technique of bone marrow collection under general anesthesia in the operating room, freezing, storage, thawing, and reinfusion in the patient has been described previously.

The visual integrity of the bone marrow was verified before and during its collection on multiple marrow aspirations and biopsies. A volume of 1 L was obtained from each patient ($1\text{-}3 \times 10^8$ nucleated medullary cells/kg; 4278-32185 CFUc/kg; 9495-50632 BFUe/kg). The marrow was divided into nine samples of 100 ml each in bags made of Teflon-Capton (Gambro DF 1200, Gambro-Hechingen, FRG), and the remaining 100 ml was used for control studies. Three hundred milliliter was immediately frozen without prior manipulation and kept as back-up marrow, and 600 ml was incubated in the presence of 10^{-2} M ammonium chloride and immunotoxin in an incubator at 37°C with constant gentle shaking.

The dose of immunotoxin was 10^{-9} M for Patients 1 and 2 and 10^{-8} M for Patients 3 and 4. Incubation time was 4 hours for Patient 1 and 20 hours for Patients 2-4. These conditions were chosen as a consequence of preliminary experiments showing that a 6-log cytoreduction (evaluated by clonogenic assay) could be achieved following incubation of the immunotoxin T 101 with a leukemic cell line bearing the T 65 antigen (13).

Following incubation, the six bags of marrow were successively frozen one by one, without preliminary wash-

TABLE 1.—Clinical situation of patients*

Patient No.	Age (yrs)/sex	Initial diagnosis	Immunological typing	Treatment prior to autografting	Situation at time of autografting	High-dose therapy by ABMT
1	25/F	Lymphoblastic T-cell lymphoma, stage III	E rosettes positive 10-2 positive 9-6 positive D 47 positive	M-BACOD	1st remission	CY (60 mg/kg/day × 2) + TBI
2	38/F	Lymphoblastic T-cell lymphoma, state IV; initial bone marrow involvement	E rosettes positive OKT 4 positive OKT 6 positive OKT 8 positive OKT 11 positive	BACOD; meningeal prophylaxis	Subdiaphragmatic relapse	CY (60 mg/kg/day × 2) + TBI
3	17/M	Lymphoblastic T-cell lymphoma, stage III	E rosettes positive OKT 11 positive OKT 6 positive OKT 4 & OKT 8 positive	BACOD; meningeal prophylaxis	1st remission	CY (60 mg/kg/day × 2) + TBI
4	17/M	Acute lymphoblastic T-cell leukemia	OKT 3 positive OKT 11 positive T 101 positive 39200, T 65 antigens/cell	CY-DN-VCR, ARA-C, LASP, intrathecal methotrexate, irradiation of brain (15 Gy)	2nd remission; previous meningeal involvement with facial nerve paralysis	CY (60 mg/kg/day × 2) + ARA-C (3 g/m² × 3) + TBI

*M-BACOD = methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dacarbazine; CY = cyclophosphamide; TBI = total-body irradiation 10 Gy (lung shielding at 6 Gy for Patients 1 and 2 and at 8 Gy for Patients 3 and 4); DN = daunorubicin; VCR = vincristine; ARA-C = cytarabine, and LASP = L-asparaginase.

ing. The freezing medium consisted of TC 199 medium with 20% dimethyl sulfoxide and 10% compatible human serum; it was added volume for volume to each bag just before freezing. Freezing was done in a Cryoson BV-4 apparatus (Cryoson, Midden Beemster, Holland), which was modified by us to specifically annul the heat buildup induced by fusion and to maintain the cooling rate beyond the transition phase (< 5°C/min) (14-17).

The enumeration of CFUc (colony-forming units, culture) by the technique of Pike and Robinson (18) with an underlying leukocyte layer, and the enumeration of BFUe (burst-forming units, erythroid) in methylcellulose by the technique of Iscove and Sieber (19) were done routinely in all of the patients at each stage of the investigation: (*a*) on the fresh marrow; (*b*) after incubation in the presence of immunotoxin before freezing; and (*c*) after thawing. All results were expressed in numbers of colonies per milliliter of marrow.

The amount of marrow infused was determined directly on the thawed marrow. In parallel with the above studies, samples of marrow from each individual patient were used to study the sensitivity of progenitor CFUc and BFUe to ammonium chloride and immunotoxin in increasing doses from 10^{-9} M up to 10^{-6} M for incubation periods of variable duration. Results of these tests are reported elsewhere (12).

Chemotherapy, Total-Body Irradiation, and Marrow Autografts

Heavy consolidation treatment in the first three patients consisted of cyclophosphamide (60 mg/kg × 2) followed in 48 hours by total-body irradiation with 10 Gy, with a dose rate at the source of < 6 cGy/minute, and lung shielding at 6 Gy. Patient 4 received a single dose of cyclophosphamide associated with a high dose (9 g/m^2) of cytarabine, followed by total-body irradiation with 10 Gy and lung shielding at 8 Gy. Prevention of hemorrhagic cystitis was attempted with mesna (60% of the dose of cyclophosphamide/day of administration) for all of the patients.

The bags of marrow were thawed at the patient's bedside and given immediately, each one in < 10 minutes, at the rate of two bags a day for 3 consecutive days, along with forced diuresis of 3 L/m^2 to assure good elimination of hemoglobin released from lysed erythrocytes.

The autologous transplantation was done in a sterile room. No gastrointestinal sterilization was carried out, but two patients received polyvalent gammaglobulins (CNTS, Paris) in a dose of 0.4 g/kg every 10 days, and all received a dose of ketoconazole sufficient to maintain an effective serum level > 1 μg/ml. Parenteral hyperalimentation was instituted if needed. All blood products were irradiated. The hematological restitution of the patient was followed daily on hemograms, and weekly on bone marrow aspiratons, biopsies, and cultures for BFUe and CFUc. During the stay in the sterile unit, surveillance was strict, and particular attention was given to secondary effects likely to result from the injection of the immunotoxin present in the bags of thawed marrow. Renal function was monitored daily; liver and hemostasis were tested twice a week. Following discharge, the patients were seen in the clinics every 15 days. The reconstitution of the lymphocyte subpopulations of the peripheral blood was studied by immunofluorescence technique, and particular attention was devoted to the helper/suppressor ration (OKT 4/OKT 8).

Analysis of Immunotoxin Circulating In Vivo

Blood samples were obtained from every patient at intervals varying from 5 minutes after administration of the first bag to 5 minutes after the final bag (47 hours after time zero). The serum level of immunotoxin was determined by radioimmunological technique, and the results were given in molar concentration of immunotoxin on the basis of a rate of coupling of 2.5 A chains per molecule of antibody.

RESULTS

Effect of Immunotoxin on Hematopoietic Precursors and Amounts of Marrow Administered (table 2)

In the four patients in this preliminary study, the recovery of hematopoietic precursors at the end of incubation with immunotoxin varied between 54% ± 8% and 255% ± 62% for CFUc and 27% ± 8% and 121% ± 34% for BFUe.

Increasing the incubation time (from 4 to 20 hours) and/or the dose of immunotoxin (from 10^{-9} to 10^{-8} M) did not cause any decrease in the recovery of CFUc, but was associated with a slight decrease for the BFUe. Progenitor cell recoveries after cryopreservation were poor: from 15% ± 4% to 80% ± 15% for CFUc and from 33% ± 22% to 47% ± 24% for BFUe. As a result, the doses of marrow given for the autograft were often only a small fraction of the doses treated initially: from 10% to 48% in CFUc, except for Patient 2 where the injected dose was almost twice that of the initially incubated one, and from 2% to 92% for BFUe.

Autologous Bone Marrow Transplantation: Hematopoietic Recovery (table 3)

In the four patients, the successful take of the graft was verified on the hemogram by the presence of proerythroblasts and basophilic erythroblasts between Days 7 and 9. Counts of 10^9/L leukocytes and 5 × 10^8/L polynuclear neutrophils were found from Days 15 to 31 and Days 23 to 32, respectively. The appearance of reticulocytes (> 0.1%) occurred between Days 14 and 26. A plate-

TABLE 2.—In vitro treatment of marrow by immunotoxin T 101

Patient No.	Doses of treated marrow ($\times 10^5$)		Immunotoxin dose/duration of incubation	Recovery after incubation (%)*		Recovery after cryopreservation (%)†		Dose of marrow infused/kg	
	CFUc	BFUe		CFUc	BFUe	CFUc	BFUe	CFUe	BFUe
1	9.56	5.43	10^{-9} M/4 hrs	54 ± 8	121 ± 34	38 ± 21	34 ± 3	4493	5013
2	1.55	4.13	10^{-9} M/20 hrs	255 ± 62	36 ± 16	80 ± 15	—	5236	—
3	5.42	4.14	10^{-8} M/20 hrs	96 ± 19	37 ± 24	28 ± 3	47 ± 24	4043	5871
4	9.57	21.72	10^{-8} M/20 hrs	118 ± 16	27 ± 8	15 ± 4	33 ± 22	1436	573

*Calculated by dividing the total No. of progenitors (CFUc or BFUe)/ml of marrow following incubation by the total No. of progenitors/ml of marrow before incubation.

†Calculated in relation to progenitors CFUc and BFUe present at the end of incubation with immunotoxin.

let count of $> 50 \times 10^9$/L was noted for Patients 1-3 between Days 22 and 47. Patient 4 developed peripheral thrombocytopenia and liver veno-occlusive disease, presumably related to pregraft conditions. In this patient, the presence of megakaryocytes on marrow aspirations, however, made it possible to confirm the recovery of thrombopoiesis. The number of circulating lymphocytes reached 10^9/L between Days 22 and 96. In two cases (Patients 1 and 4), parallel with the development of intercurrent infection, the circulating lymphocyte counts secondarily fell and remained $< 0.5 \times 10^9$/L until death. In all cases, the ratio of T "helper"/T "suppressor-cytotoxic" remained low (< 0.5) up to Day 90 when the study ended.

Evolution, Side Effects, and Circulating Level of Immunotoxin

In the immediate period of aplasia in the sterile unit, no special side effects were noted that might be related directly or indirectly to the in vitro incubation with immunotoxin. Administration of marrow without previous washing did not give any reaction. Two months after the autograft, Patient 1 developed a first infection of undetermined origin, probably viral, which was characterized by a mononucleosis syndrome, meningitis, and lesion of the posterior columns of the spinal cord. This was followed by a reversible *Pneumocystis carinii* pulmonary infection, generalized herpes zoster, and, finally, extensive pulmonary fibrosis leading to death, while in complete remission of the lymphoma, 5 months after the autograft. Lymphocytosis remained at $< 0.5 \times 10^9$ cells/L during the last month of the illness. Patient 2, treated during partial remission, achieved complete remission for 3 months but then relapsed in the same locations and eventually died 7 months later. Patient 3 remains in complete remission at this time, 11 months after the autograft. Three months after the autograft, Patient 4, in complete persisting remission, first developed liver veno-occlusive disease with ascites and collateral venous circulation, which resolved spontaneously, then developed a massive cytomegalovirus pneumopathy which proved fatal on Day 120, in spite of treatment with acyclovir and alpha interferon. During the final month, lymphocytosis remained at $< 0.5 \times 10^9$ cells/L.

The analyses of immunotoxin in the circulation showed concentrations $< 10^{-9}$ M (table 4).

DISCUSSION

Ricin is a vegetable toxin composed of two polypeptide subunits A and B. It poisons the cell in a sequential manner: the B fraction, endowed with recognition properties, is able to attach itself to galactoside residues present on the surface of all animal cells, thus allowing the passage of chain A into the cytoplasm by the intermediary action of phagolysosomes. Eventually, the A chain is fixed on the ribosomal apparatus and causes cell death by inhibition of protein synthesis. Ricin toxicity is consequently potent and nonspecific (20), while cell destruction from immunotoxin is specific.

Immunotoxin T 101 is a hybrid molecule composed of the murine monoclonal antibody T 101 coupled by a disulfur bridge to chain A of ricin (21-23). The monoclonal antibody T 101, an immunoglobulin of the class IgG2a, recognizes the surface antigen T 65 with a molecular weight of 65,000 daltons, which is present on mature and immature T cells (24), tumor T cells, and B cells of chronic lymphoid leukemia (25), but which is absent from granular and erythroblastic progenitors (11). Fixation of antibody T 101 on antigen T 65 leads to internalization of chain A. Judged by protein synthesis, T 101 toxicity is much greater than that of chain A of ricin alone for tumor cells of the CEM lineage (factor of specificity). Its effectiveness is increased in vitro in the presence of ammonium chloride, 10^{-2} M, which protects the immunotoxin against intralysosome neutralization (26,27). Under these conditions, a factor of specificity of 300,000 can be obtained, with incubation time reduced to 8 hours (23). The cytolytic effectiveness of the immunotoxin is proportional to the number of antigenic sites on the target cells (28).

An immunotoxin made according to the same principles, but using purified polyvalent rabbit anti-mouse immunoglobulins, recognizing antigen determinants B, was tested in an autograft model against the BCL 1 murine tumor (29). In this model, the administration to irradiated mice of healthy marrow contaminated with 15% tumor cells, but previously incubated in the presence of

TABLE 3.—Hematopoietic recovery after autograft of marrow treated by immunotoxin

Patient No.	Aspiration Day showing— Evidence of engraftment on marrow	Leukocyte count $> 10^9$/L	Polymorphonuclear neutrophil count $> 5 \times 10^8$/L	Reticulocyte count > 0.1%	Platelet count $> 5 \times 10^9$/L	Lymphocyte count $> 10^9$/L	Helper/suppressor ratio		Medullary progenitors between days 15 and 30/ml		No. of support transfusions		Evolution
							Ratio	Day	CFUc	BFUe	Rbcs	Platelets	
1	9	15	23	18	24	22 (subsequent fall)	0.14 0.35	26 67	7	0	11	5	Died at 5 mos from acute immunodeficiency disease syndrome + pulmonary fibrosis (lymphocyte count $< 5 \times 10^8$/L)
2	8	31	32	26	47	96	0.5 0.42 0.48	26 67 105			6	10	Local recurrence at 3 mos; alive at 8+ mos
3	8	23	30	14	22	60			13		4	3	Persisting remission at 6+ mos
4	7	21	25	19		58 (subsequent fall)	0.05	70	159	681	21	40	Died on Day 120, in persisting complete remission, from liver veno-occlusive disease + cytomegalovirus pneumonitis (lymphocyte count $< 5 \times 10^8$/L)

immunotoxin, permitted the recovery of hematopoiesis and, in 80% of cases, no tumor proliferation for the rest of the animal's life. Nevertheless, transfer of leukemia to other irradiated healthy animals remained possible with minced tissue from autografted animals. These results indicate that the reduction of the tumor load achieved with immunotoxin was in the range of 4-5 logs (30). In man, monoclonal antibody T 101 has been administered to patients with chronic lymphoid leukemia and cutaneous T-cell lymphoma (31,32) with partial antitumor effectiveness, most often transitory, and some side effects such as pulmonary and allergic reactions attributed to leukoagglutination, especially in the case of rapid infusion of the antibody. An important immunomodulation phenomenon was observed in the course of these therapeutic trials. Some allografts and autografts have been realized with marrow incubated in vitro in the presence of monoclonal T antibodies (33-35) or anti-T immunotoxins (36), demonstrating the feasibility of the technique and the absence of hematopoietic toxicity from these manipulations. Yet, we do not know of any marrow autografts incubated with antibody T 101, and our report is the first concerning the use of immunotoxin T 101-A chain, potentiated by ammonium chloride. As compared to the use of monoclonal antibodies and complement, immunotoxins offer the following advantages: (*a*) it is not necessary to select and store a large volume of baby rabbit complement effective against tumor cells but still devoid of toxicity against normal progenitor cells; and (*b*) it is not necessary to manipulate the marrow either before or after the addition of immunotoxin. Furthermore, reinfusing immunotoxin with the unwashed marrow may produce an additional tumor cytoreduction in vivo, with no potential toxicity, the dose infused (0.04 mg) being by far (1/500) smaller than the LD50 evaluated in rats (20 mg/kg) (37).

This clinical trial followed previous in vitro investigations which had shown absence of toxicity for CFUc and BFUe of 10^{-2} M of ammonium chloride and of immunotoxin in concentrations inferior to 10^{-6} M for incubation periods of ⩽ 20 hours (12). Also, in similar conditions, a 6-log tumor cytoreduction (assessed by clonogenic assay) could be measured when T 65 + leukemic cells were incubated with T 101 immunotoxin (13). In conformity with the results of these studies, the doses of immunotoxin which we used in our clinical trial were much less than the known toxic threshold for the progenitors. In vitro studies had also revealed a weakening of precursors due to freezing following incubation. As predicted, recovery after cryopreservation was poor, but a dose of marrow sufficient for doing the autograft (> 1000 CFUc/kg) (15) was obtainable in every case.

TABLE 4.—Serum levels of immunotoxin T 101 in Patient 4

Blood sample interval*	Immunotoxin expressed as chain A (ng/ml)	Immunotoxin (M)
5 mins	6.5	2.6×10^{-10}
15 mins	5.3	2.12×10^{-10}
60 mins	2.5	1×10^{-10}
93 mins	1.2	0.48×10^{-10}
21 hrs 40 mins	—	—
24 hrs 13 mins	2.6	1.04
27 hrs 6 mins	4.4	1.76
46 hrs 28 mins	—	—
48 hrs 20 mins	3.8	1.52
70 hrs 45 mins	—	—

* Time after administration of bags of marrow.

Engraftment occurred in all patients, including Patient 4, in whom the hemogram showed the early presence of megakaryocytes, in spite of peripheral thrombocytopenia.

Recovery to a wbc count of 1000/mm^3 occurred within usual periods (17). However, recovery of the neutrophil count to 500/mm^3 occurred at a median of 28 days, a somewhat longer period than that observed in a similar population of patients with non-Hodgkin's lymphoma receiving total-body irradiation plus ABMT of nontreated marrow (median, 18 days; range, 12-37) (16). Therefore, the possibility that engraftment was somewhat delayed cannot be ruled out. Also, we had some concern about the recovery of lymphocytes, which was slow in all patients, and, in two patients, was followed by a severe secondary lymphopenia associated with the development of lethal infections.

Since the purpose of our study was to check the absence of toxicity of immunotoxin for hematopoietic progenitors, the four patients included in this trial were selected only in view of poor prognosis criteria and presence of T markers on tumor cells. The number of T 65 antigens on tumor cells and their in vitro sensitivity to immunotoxin T 101 were not studied in vitro except for one patient. Therefore, and in view of the small number of patients treated, the antitumor effectiveness of autografting with marrow incubated with immunotoxin T 101 cannot be fully assessed and requires further trials.

We conclude from this preliminary report that autologous bone marrow transplantation with marrow incubated in the presence of immunotoxin is feasible without toxicity from the immunotoxin for the erythropoietic, granular, or platelet progenitors, and without direct or indirect organ toxicity related to its reinfusion with the treated marrow. The nature of the monoclonal antibody used to support the toxin is such that it could have possible repercussions on other cell populations. In this regard, the repercussion of immunotoxin T 101 on the lymphocyte precursors deserves particular attention.

REFERENCES

1. GORIN NC. For the working party on autologous bone marrow transplantation of the European Bone Marrow Transplantation group: autologous bone marrow transplantation for acute leukemia in Europe. Exp Hematol 12 (suppl 15):123-125, 1984.
2. HERVE P, PHILIP T, FLESCH M, ET AL. Intensive cytoreductive regi-

men and autologous bone marrow transplantation in leukemia. Present status and the future, a review. Eur J Cancer Clin Oncol 19: 1043-1052, 1983.
3. APPELBAUM FR, DEISSEROTH AB, GRAW RG, ET AL. Prolonged complete remission following high dose chemotherapy of Burkitt's lymphoma in relapse. Cancer 41:1059-1063, 1978.
4. GORIN NC, NAJMAN A, DOUAY L, ET AL. Autologous bone marrow transplantation in the treatment of poor prognosis non-Hodgkin's lymphomas. Eur J Cancer Clin Oncol 20:217-225, 1984.
5. PHILIP T, BIRON P, MARANINCHI D, ET AL. Role of massive chemotherapy and autologous bone marrow transplantation in non-Hodgkin's malignant lymphomas. Lancet 1(8373):391, 1984.
6. PHILLIPS GL, HERZIG RH, LAZARUS HM, ET AL. Treatment of resistant malignant lymphoma with cyclophosphamide, total body irradiation and transplantation of cryopreserved autologous marrow. N Engl J Med 310:1557-1561, 1984.
7. BARTLE R, FRISCH B, BURKHARDT R, ET AL. Assessment of bone marrow histology in the malignant lymphomas (non-Hodgkin's): correlation with clinical factors for diagnosis, prognosis classification and staging. Br J Haematol 51:511-530, 1982.
8. FOUCAR K, MCKENNA RW, FRIZZERA G, ET AL. Incidence and patterns of bone marrow involvement by lymphoma in relationship to the Lukes-Collins classification. Blood 54:1417-1422, 1979.
9. BAST RC, RITZ J, LIPTON JM, ET AL. Elimination of leukemic cells from human bone marrow using monoclonal antibody and complement. Cancer Res 43:1389-1394, 1983.
10. DOUAY L, GORIN NC, GEROTA I, ET AL. In vitro treatment of leukemic bone marrow for autologous transplantation. Exp Hematol 10(suppl 12):113-122, 1982.
11. TAETLE R, and ROYSTON I. Human T cell antigens defined by monoclonal antibodies. Absence of T 65 on committed myeloid and erythroid progenitors. Blood 56:943-946, 1980.
12. DOUAY L, GORIN NC, CASELLAS P, ET AL. Preclinical study of immunotoxin T 101: application to in vitro therapy prior to autologous bone marrow transplantation. Cancer Res, in press.
13. CASELLAS P, CANNAT X, GROS O, ET AL. Optimal elimination of leukemic T cells from human bone marrow with T 101 ricin A chain immunotoxin. Blood, in press.
14. DOUAY L, GORIN NC, and DUHAMEL G. Rôle critique de la vitesse de refroidissement au-delà de la libération de la chaleur de fusion, pour la conservation des cellules souches hématopoiétiques. C. R. Acad. Sc. Paris, 1982, 294, Série III, pp 103-106.
15. DOUAY L, GORIN NC, DAVID R, ET AL. Cryopreservation of hematopoietic progenitors. The interest for autologous bone marrow monitoring. Exp Hematol 11:172, 1983.
16. GORIN NC, HERZIG G, BULL MI, ET AL. Long term preservation of bone marrow and stem cell pool in dogs. Blood 51:257-265, 1978.
17. GORIN NC, DAVID R, STACHOWIAK J, ET AL. High dose chemotherapy and autologous bone marrow transplantation in acute leukemias, malignant lymphomas and solid tumors. A study of 23 patients. Eur J Cancer 17:557-568, 1981.
18. PIKE BL, and ROBINSON WA. Human bone marrow colony growth in agar gel. J Cell Physiol 76:77-84, 1970.
19. ISCOVE BL, and SIEBER F. Erythroid progenitors in mouse bone marrow detected by macroscopic colony formation in culture. Exp Hematol 3:23, 1975.
20. OLSNES S, REFSNES K, and PIHL A. Mechanism of action of the toxic lectins abrin and ricin. Nature 249:627, 1974.
21. BLYTHMAN HE, CASELLAS P, GROS O, ET AL. Immunotoxins: hybrid molecules of monoclonal antibodies and a toxin subunit specifically kill tumour cells. Nature 290(5802):145-146, 1981.
22. JANSEN FK, BLYTHMAN HE, CARRIERE D, ET AL. High specific cytotoxicity of antibody-toxin hybrid molecules (immunotoxins) for target cells. Immunol Lett 2:97-102, 1980.
23. JANSEN FK, BLYTHMAN HE, CARRIERE D, ET AL. Immunotoxins hybrid molecules combining high specificity and potent cytotoxicity. Immunol Rev 62:185-216, 1982.
24. ROYSTON I, MADJA JA, BAIRD SM, ET AL. Monoclonal antibody specific for human T lymphocytes: identification of normal and malignant T cells. Blood 54(suppl 1):106a, 1979.
25. ROYSTON I, MADJA JA, BAIRD SM, ET AL. Human T cell antigens defined by monoclonal antibodies: the 65,000 dalton antigen of T cells (T 65) is also found on chronic lymphocytic leukemia cells bearing surface immunoglobulin. J Immunol 125:725-731, 1980.
26. CASELLAS P, BOURRIE BJP, GROS P, ET AL. Kinetics of cytotoxicity induced by immunotoxins: enhancement by lysosomotropic amines and carboxylic ionophores. J Biol Chem 259:9359-9364, 1984.
27. CASELLAS P, BROWN JP, GROS O, ET AL. Human melanoma cells can be killed in vitro by an immunotoxin specific for melanoma-associated antigen p 97. Int J Cancer 30:437-443, 1982.
28. CARAYON P. Expression antigénique et efficacité des immunotoxines: mémoire pour le diplome d'ingénieur CNAM spécialité biologie: Conservatoire National des Arts et Métiers. Centre Associé du Languedoc-Roussillon, Nov 2, 1983.
29. KROLICK KA, UHR JW, and VITETTA ES. Selective killing of leukaemia cells by antibody-toxin conjugates: implications for autologous bone marrow transplantation. Nature 295:604-605, 1982.
30. VITETTA ES. The use of immunotoxins to kill neoplastic B cells. *In* Minimal Residual Disease in Acute Leukemia (Lowenberg B, and Hagenbeek A, eds). Boston, The Hague, Dordrecht, Lancaster, Martinus Nijhoff Publishers, 1984, pp 223-232.
31. DILLMAN RO, SHAWLER DL, SOBOL RE, ET AL. Murine monoclonal antibody therapy in two patients with chronic lymphocytic leukemia. Blood 59:1036-1045, 1982.
32. FOON KA, SCHROFF RW, BUNN PA, ET AL. Effects of monoclonal antibody therapy in patients with chronic lymphocytic leukemia. Blood 64:1085-1093, 1984.
33. HERVE P, RACADOT E, FLESCH M, ET AL. T cell depletion using monoclonal pan T antibody combination and complement-mediated cytolysis for prevention of graft versus host disease. Tenth Annual Meeting of the European Cooperative Group for Bone Marrow Transplantation, Sierra Nevada (Granada), Spain, Jan 30-Feb 2, 1984. Exp Hematol 12(suppl 15):59-60, 1984.
34. KAIZER H, LEVY R, BROVALL C, ET AL. Autologous bone marrow transplantation in T cell malignancies. A case report involving in vitro treatment of marrow with a pan T cell monoclonal antibody. J Biol Response Mod 1:233-243, 1982.
35. REINHERZ EL, GEHA R, RAPPAPORT JM, ET AL. Reconstitution after transplantation with T lymphocyte depleted HLA haplotype-mismatched bone marrow for severe combined immunodeficiency. Proc Natl Acad Sci USA 79:6047-6051, 1982.
36. FILIPOVICH AH, VALLERA DA, YOULE RJ, ET AL. Ex vivo treatment of donor bone marrow with anti-T cell immunotoxins for prevention of graft versus host disease. Lancet 1:469-471, 1984.
37. MAZUE G. Expertise toxicologique préliminaire de l'immunotoxine SR 41322. Centre de Recherches Clin Midy, Montpellier, France.

Phase I–II Trial With Cisplatin and 5-FU in Recurrent Head and Neck Cancer: An Effective Outpatient Schedule[1]

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Thirty patients with squamous cell carcinoma of the head and neck were treated with a combination of cisplatin (20 mg/m^2) and 5-FU (400–200 mg/m^2) by iv push on Days 1–5 every 21 days. All patients but two had relapsed disease. Twenty-seven patients were evaluable for response: there were four complete responses and 12 partial responses (objective response rate, 59.2%). The response rate falls to 53.3% if it is calculated on the total number of patients entered. Twenty-seven patients were evaluable for toxicity: myelosuppression occurred in ten patients (37%), while renal toxicity, nausea and vomiting, and stomatitis were quite rare and moderate. These results look encouraging and suggest the need for further studies. [Cancer Treat Rep 69:961–964, 1985]

The use of cisplatin (CDDP) in association with continuous-infusion 5-FU is one of the more recent and interesting developments in the treatment of squamous cell carcinoma of the head and neck. The original schedule (1) requires hospitalization, and subsequent adaptations allowing outpatient administration have produced disappointing results (2,3). On the other hand, both the cost of hospitalization and the lack of facilities justify further attempts to characterize an effective outpatient schedule. We have studied a different combination of CDDP and 5-FU in outpatients with advanced or relapsed head and neck cancer.

MATERIALS AND METHODS

Between November 1983 and July 1984, 30 patients with recurrent (28 patients) or advanced unresectable (two) squamous cell carcinoma of the head and neck were treated with CDDP and 5-FU. Patient characteristics are shown in table 1. All patients but one (No. 16) had received prior chemotherapy in a first-line program. Pretreatment evaluation included physical examination, chest x-ray, creatinine clearance, and other relevant scans or roentgenograms. Informed consent was obtained and histologic confirmation of diagnosis was required. The chemotherapy schedule was as follows: CDDP (20 mg/m^2 for 5 consecutive days) was administered after prehydration (500 ml of 5% dextrose + 5 mEq/L of KCl over a 30-minute infusion). CDDP was given if diuresis was $\geq$ 200 ml/30 minutes. Posthydration consisted of 1.5 L of 5% dextrose plus 5 mEq/L of KCl. Methylprednisolone (125 mg iv) was given at the time of CDDP administration for control of nausea and vomiting. 5-FU was administered by iv push after the end of posthydration. Initially, the dosage was 400 mg/m^2, but it was reduced to 200 mg/m^2 due to hematologic toxicity encountered in the first patients. Criteria for response were the following. Complete response (CR) was defined as the complete disappearance of all clinically detectable disease. Partial response (PR) was defined as a 50% reduction in the sum of the products of the longest diameters of all measurable lesions, without appearance of new lesions. In evaluable patients, PR was defined as an improvement of two grades or normalization in ECOG PS scale, with a grossly reduced mass. Stable disease was defined as a $< 50\%$ reduction in the sum of the products of the longest diameters of all measurable lesions or no change in tumor measurement, lasting > 2 months. Progressive disease was defined as a $\geq 25\%$ increase in the sum of the product of the longest diameters of tumor size or the occurrence of any new lesion.

RESULTS

Of the 30 patients entered, 27 are evaluable for both response and toxicity: two patients who had early death and three who had changes of therapy due to

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TABLE 1.—Characteristics of patients and response

Patient No.	Sex	Age (yrs)	ECOG PS*	Lesion†	Primary site	Prior therapy‡			5-FU dose (mg/m^2)	No. of courses given	Toxicity		Response§	Time to disease progression (wks)	Survival (wks)
						CT	S	RT			Renal (mg/100 ml)	Hematologic ($cells/mm^3$)			
1	M	73	0	M	Oropharynx	—	—	—	400	1	—	—	NE (lost to FU)	—	—
2	M	54	1	M	Oropharynx	VBA	—	Yes	400	1	—	—	NE (lost to FU)	—	—
3	M	57	1	M	Larynx	—	—	Yes	400	1	—	—	NE (lost to FU)	—	—
4	F	62	0	M	Oral cavity	—	Yes	—	400	1	—	Wbcs, 800; platelets, 15,000	Failure due to toxicity (change of CT)	—	28
5	M	73	1	M	Nasopharynx	—	Yes	Yes	400	1	Serum creatinine, 2.2	—	Failure due to toxicity (change of CT)	—	12
6	M	54	1	M	Larynx	VBA	—	Yes	400	1	Serum creatinine, 2.0	—	Failure due to toxicity (change of CT)	—	15
7	M	73	3	M	Oral cavity	—	—	Yes	400	1	—	Platelets, 15,000	Failure due to toxicity (death)	—	3
8	M	61	1	M	Oral cavity	—	Yes	Yes	400	1	—	Wbcs, 200; platelets, 20,000	Failure due to toxicity (death)	—	3
9	M	56	1	M	Oral cavity	VBA	—	Yes	400	4	—	—	SD	16	25
10	M	59	3	M	Hypopharynx	CAFB	—	Yes	400	2	—	—	PR	4	4
11	F	62	1	M	Oral cavity	—	Yes	—	400	4	—	Platelets, 80,000	PR	31	37
12	F	70	1	M	Maxillary sinus	—	Yes	Yes	400	5	—	Wbcs, 1350; platelets, 52,000	CR	23	31
13	F	52	1	M	Oropharynx	VBA	—	Yes	400	3	Serum creatinine, 1.8	—	PR	8	32
14	F	61	0	E	Nasopharynx	—	—	—	400	5	—	Wbcs, 2280; platelets, 52,000	CR	40+	40+
15	M	34	1	M	Oropharynx	—	Yes	—	400	4	—	—	PR	28	34+
16	M	73	1	M	Oropharynx	MtAF, VH	—	Yes	400	4	Serum creatinine, 1.9	Wbcs, 790	PR	42+	42+
17	M	52	1	E	Nasopharynx	VBA	—	Yes	400 200	2 3	—	Wbcs, 2400	PR	29+	29+
18	M	61	3	E	Larynx	VBA	—	—	400 200	2 2	—	Wbcs, 1700	CR	16	31+
19	M	54	1	M	Oropharynx	—	Yes	Yes	400 200	6 1	— —	Wbcs, 2250 —	PR	29	29
20	M	58	0	M	Larynx	CAFB	—	Yes	200	4	—	—	PR	16	18+
21	M	34	2	M	Nasopharynx	VBA	—	Yes	200	2	—	—	P	—	4

22	M	61	1	M	Hypopharynx	—	Yes	Yes	200	5	—	—		PR	20	23+
23	M	53	1	M	Oropharynx	CAFB	Yes	Yes	200	4	—	—		SD	24	24+
24	M	39	1	E	Nasopharynx	VBA	Yes	Yes	200	5	—	—		CR	27+	27+
25	F	55	3	M	Oropharynx	—	—	Yes	200	5	—	—		PR	19	19
26	M	49	0	M	Oral cavity	—	Yes	—	200	2	—	—		P	—	23
27	M	56	1	M	Maxillary sinus	—	—	Yes	200	4	—	—		SD	12	15
28	M	71	0	M	Larynx	—	Yes	Yes	200	4	—	—		PR	24	30+
29	M	60	3	M	Oropharynx	—	Yes	—	200	4	—	—		PR	33	34+
30	M	56	1	M	Larynx	—	—	Yes	200	2	—	—		PD	—	6

* Eastern Cooperative Oncology Group performance scale.
†M = measurable; and E = evaluable.
‡CT = chemotherapy; S = surgery; RT = radiotherapy; V = vincristine; B = bleomycin; A = methotrexate; C = cyclophosphamide; F = 5-FU; Mt = mitomycin; and H = hydroxyurea.
§NE = not evaluable; FU = follow-up; SD = stable disease; and P = disease progression.

toxicity after the first chemotherapy course were classified as failures to therapy. Three patients were lost to follow-up after the first chemotherapy course and therefore were not evaluable. The overall response rate was 59.2% (four CRs and 12 PRs); three patients had stable disease and eight had treatment failures. The response rate falls to 53.3% if it is calculated on the total number of patients entered. Response by prior therapy, PS, and site of primary is shown in table 1. The median survival was 28+ weeks; it was not reached at 40 weeks in patients achieving CR, at 37+ weeks in those achieving PR, or at 15 weeks in those who failed to respond. Median time to disease progression was 24+ weeks: 23+ weeks in patients achieving CR and 24+ weeks in those achieving PR. Three patients had stable disease lasting 12, 16, and 24 weeks. Table 1 shows reported survival and time to disease progression of each patient entered in the study. Ten of 27 patients had hematologic toxicity; two patients died after the first course of chemotherapy, Patient 8 from bronchopneumonia (wbc count, 0.2×10^3) and Patient 7 from gastric hemorrhagia (platelet count, 15×10^3). Three patients had life-threatening leukopenia or severe thrombocytopenia (Patients 4, 12, and 16) after the first course of chemotherapy, and for one of them it was necessary to change therapy. Five patients had moderate leukopenia (wbc count, $1.5\text{–}2.5 \times 10^3$) or thrombocytopenia (platelet count, $50\text{–}95 \times 10^3$). In all patients, hematologic toxicity was related to the higher dosage of 5-FU; no further leukopenia or thrombocytopenia was detected after the 5-FU dosage reduction. Renal toxicity appeared in four patients (11.6%), and two of them changed therapy after the first course (Patients 5 and 6). One patient developed severe stomatitis, but he continued the therapy after some delay; nausea and vomiting occurred in seven patients (25.9%), but it was usually moderate and managed with antiemetics.

DISCUSSION

Chemotherapy is only moderately effective in palliating recurrent squamous cell carcinoma of the head and neck. The most effective agents include few drugs, like methotrexate, CDDP, bleomycin, and 5-FU, but response rates are usually low and most responses are partial and of short duration (4). The introduction of CDDP combined with 5-FU infusion in the management of recurrent epidermoid cancer of the head and neck by Kish et al (5) seems to improve the prognosis of recurrent disease, but the scheduling of that combination requires hospitalization. We have studied a different scheduling of CDDP and 5-FU, easily adaptable to outpatients, reducing the cost of hospitalization and improving patient compliance to the therapy. Our preliminary analysis seems to show a very similar efficacy

of this new scheduling compared to the results reported by Kish et al (5). In our series, patients had received very heavy prior treatment: 11 of 27 patients had received prior chemotherapy and in most patients (ten of 11) it was combined with irradiation and/or surgery. Moreover, after reducing the dosage of 5-FU, toxicity was substantially reduced from that reported by Kish et al (1,5). The fractionation of CDDP dosage in 5 consecutive days is also under study at Wayne State University; Kish et al (6) reported encouraging preliminary results using CDDP (40-30 mg/m^2) with hypertonic saline for 5 consecutive days combined with 5-FU continuous infusion. In conclusion, both the responses reached and the toxicity encountered support a more detailed evaluation of this combination.

REFERENCES

1. KISH J, DRELICHMAN A, JACOBS J, ET AL. Clinical trial of cisplatin and 5-FU infusion as initial treatment for advanced squamous cell carcinoma of the head and neck. Cancer Treat Rep 66:471-474, 1982.
2. KISH J, ENSLEY J, WEAVER A, ET AL. Superior response rate with 96 hour 5-FU infusion vs. 5-FU bolus combined with cisplatinum in a randomized trial for recurrent and advanced squamous head and neck cancer. Proc ASCO 25:179, 1984.
3. MERLANO M, CONTE PF, TATAREK R, ET AL. Ineffectiveness of 5-fluorouracil and cisplatin as second-line chemotherapy in head and neck cancer. Tumori 70:267-269, 1984.
4. JACOBS C. Use of methotrexate and 5-FU for recurrent head and neck cancer. Cancer Treat Rep 66:1925-1928, 1982.
5. KISH JA, WEAVER A, JACOBS J, ET AL. Cisplatin and 5-fluorouracil infusion in patients with recurrent and disseminated epidermoid cancer of the head and neck. Cancer 53:1819-1824, 1984.
6. KISH J, ENSLEY J, JACOBS J, ET AL. Preliminary report of phase I-II evaluation of high dose *cis*-platinum (CACP) with 120 hour 5-fluorouracil infusion as induction therapy for advanced head and neck cancer (HNC). *In* Proceedings of the International Conference on Head and Neck Cancer. Baltimore, 1984, vol 97, p 110.

Phase II Study With Amsacrines (*m*-AMSA and *m*-AMSA Lactate) in Refractory Lymphomas[1,2]

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A total of 70 patients with malignant lymphomas refractory to one or more chemotherapeutic regimens were treated with iv amsacrines (*m*-AMSA and *m*-AMSA lactate). Of 58 evaluable patients, 12 had Hodgkin's disease and 46 had non-Hodgkin's lymphoma. Twenty-nine of the evaluable patients received *m*-AMSA and 29 received *m*-AMSA lactate. The amsacrines were recycled every 3 weeks. The doses of *m*-AMSA were 90–120, 70, and 25–30 mg/m²/day for 3 days, respectively. All patients treated with *m*-AMSA lactate received a single dose of 225 mg/m². In Hodgkin's disease, the response rate was 58.3% (one complete response among 12 patients), and in non-Hodgkin's lymphoma, the response rate was 30.4% (six complete responses among 46 patients). The median duration of response was 3 and 5 months, respectively. The response rate was unfavorably affected by the presence of extranodal disease and a Karnofsky performance status < 80. There was no important difference in the incidence and duration of response between *m*-AMSA and *m*-AMSA lactate. After vomiting, myelosuppression was the most frequently observed toxic effect. One patient showed an unexpected fatal bone marrow aplasia following the first course of 90 mg/m². This study indicates that *m*-AMSA and *m*-AMSA lactate are active and moderately toxic in previously treated malignant lymphomas. Thus, amsacrines could be effectively incorporated into salvage polydrug regimens. [Cancer Treat Rep 69:965–969, 1985]

The acridine derivatives were developed as potential antineoplastic agents by Cain et al (1). These compounds showed significant antitumor activity in some animal tumor systems (2), and clinical studies in man have been published since 1978 (3,4). The mechanism of action of aminoacridines or amsacrines has not been entirely elucidated; they act as intercalating agents with DNA, but other mechanisms of action may be considered for their antitumor activity. Pharmacologic studies demonstrated that aminoacridines were well-distributed in all tissues except the brain, with high drug levels in liver, kidney, and spleen. The drugs are excreted through the bile, mainly as a metabolite, and their plasma clearance is reduced in the presence of liver impairment (3,5). In man, the dose-limiting toxicity is myelosuppression. Evidence of possible cardiotoxicity has recently surfaced in the literature (6,7), and this rare side effect was reported to occur more frequently in patients previously treated with anthracyclines (8).

In the past few years, therapeutic results have confirmed some degree of activity in only refractory acute leukemias and malignant lymphomas (9–13). This paper reports the experience achieved at the Milan Cancer Institute with *m*-AMSA and *m*-AMSA lactate in Hodgkin's disease and non-Hodgkin's lymphoma refractory to one or more chemotherapeutic regimens. *m*-AMSA lactate differs from *m*-AMSA by its lack of *N,N*-dimethylacetamide.

PATIENTS AND METHODS

From November 1980 to January 1984, 70 patients with advanced and refractory malignant lymphomas were treated with amsacrines. The main patient characteristics are listed in table 1.

Patients admitted to the study were considered resistant to one or more conventional polydrug therapies. All patients received prior combination chemotherapy containing doxorubicin. In the *m*-AMSA and *m*-AMSA

[1]Received Oct 11, 1984; revised Jan 14, 1985; accepted Feb 5, 1985.

[2]Supported in part by a grant from Bristol Myers Co, New York, NY.

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TABLE 1.—Patient characteristics*

Characteristic	Total	*m*-AMSA	*m*-AMSA lactate
Total entered	70	34	36
Total evaluable (%)	58 (83)	29 (85)	29 (80.5)
Males/females	32/26	18/11	14/15
Median age in yrs	45	44	49
Hodgkin's disease	12	6	6
Non-Hodgkin's lymphoma	46	23	23
Follicular	6	3	3
Diffuse	27	13	14
Mixed	13	7	6
Disease extension			
Nodal	22	11	11
Extranodal ± nodal	36	18	18
Prior chemotherapy			
1 polydrug regimen	25	12	13
> 1 polydrug regimens	33	17	16

* Unless specified otherwise, values = No. of patients.

lactate groups, 11 of 29 and 14 of 29 patients, respectively, had previously received > 300 mg/m^2 of doxorubicin. Before starting treatment, patients were required to have measurable disease as indicator of response, an interval > 30 days since previous chemotherapy and/or radiotherapy, a Karnofsky performance status (PS) ⩾ 60, and a life expectancy > 2 months. Other requirements for eligibility were a normal blood cell count (leukocyte count ⩾ 4000/mm^3 and platelet count ⩾ 120,000/mm^3), and BUN and serum creatinine within normal limits. Patients < 15 and > 70 years of age, who had important chronic nonneoplastic disease, severe accompanying cardiovascular disease, and abnormal echocardiogram and/or ECG, were considered ineligible for this trial. In the large majority of cases, the treatment was administered in the outpatient clinic.

From November 1980 to October 1982, *m*-AMSA was administered every 3 weeks to 34 patients, utilizing different doses. In 21 patients, the dosage used was 90–120 mg/m^2, while in those heavily pretreated with radiotherapy and chemotherapy, the initial dosage was 70 mg/m^2 (four patients) or 25–30 mg/m^2 daily for 3 consecutive days (nine). From November 1982 to January 1984, *m*-AMSA lactate was administered to 36 patients at the dose of 225 mg/m^2 every 3 weeks. *m*-AMSA was infused in 250 ml of 5% glucose over 30 minutes, and *m*-AMSA lactate was infused in 500 ml of 5% glucose over 1 hour. In November 1982, *m*-AMSA was replaced by *m*-AMSA lactate. The drugs were supplied by Bristol-Myers Company, Pharmaceutical Research and Development Division, in 50-mg vials.

A minimum of two cycles had to be administered to define treatment response. In the presence of objective tumor response or static disease, therapy was continued until definite progression or response occurred. In the presence of complete remission, two additional doses were given. The maximum number of doses administered was 21. When amsacrines failed, patients were given cisplatin in the absence of specific contraindications. In the presence of grade I toxicity (leukocyte count between 3900 and 2500/mm^3 and/or platelet count between 119,000 and 80,000/mm^3), 50% of the calculated dose was administered. If grade II toxicity was evident (leukocyte count < 2500/mm^3 and/or platelet count < 80,000/mm^3), no drug was given. Blood cell counts were taken at weekly intervals, and treatment was resumed as soon as grade I toxicity was evident.

Patients were evaluated before each drug dose with physical examination and roentgenograms, complete hemogram, biochemical profile (including serum creatinine), and liver function tests. Other appropriate investigations for tumor measurement were performed in individual cases. ECG was recorded in all patients before and after treatment. Systolic time intervals, following the technique of Weissler et al (14), were recorded in the first 13 patients given *m*-AMSA lactate before therapy, and then 1 and 3 hours, respectively, after each drug administration. World Health Organization criteria for drug response were utilized in this trial (15).

RESULTS

Of the 70 patients admitted to the study, 58 (83%) were assessable for drug response. In the *m*-AMSA group, five patients were not evaluable because of inadequate treatment, ie, less than two doses. In the *m*-AMSA lactate group, seven patients were not considered evaluable for inadequate treatment (four) or lack of clearly measurable lesions (three) on retrospective evaluation.

Table 2 reports the therapeutic results related to prognostic subgroups in Hodgkin's disease and non-Hodgkin's lymphoma. Major variables unfavorably affecting the objective response rate were the presence of extranodal disease and a Karnofsky PS < 80. In non-Hodgkin's lymphoma, prior administration of more than two chemotherapy regimens was not associated with a lower response rate compared to the group of patients in whom amsacrines represented the first salvage chemotherapy. In the present series, amsacrines yielded a higher incidence of complete and partial remission in Hodgkin's disease compared to non-Hodgkin's lymphoma. The reverse occurred in the comparative evaluation of the median duration of response. However, the disproportionate number of patients between the two major lymphoma groups may, in part, account for the observed difference in the incidence and duration of response. As far as histopathologic subtypes were concerned, in Hodgkin's disease, objective

TABLE 2.—Results related to prognostic parameters*

	Hodgkin's disease†		Non-Hodgkin's lymphoma‡	
	No. of responders/ total	%	No. of responders/ total	%
Entered	7/14	50	14/56	25
Evaluable	7/12 (1)	58.3	14/46 (6)	30.4
Disease extension				
Nodal	3/3 (1)	100	9/19 (4)	47.4
Extranodal	4/9	44.4	5/27 (2)	18.5
⩽ 3 involved sites	3/5 (1)	60	6/18 (4)	33.3
> 3 involved sites	4/7	57.1	8/28 (2)	28.5
Karnofsky PS				
⩾ 80	5/6 (1)	83.3	13/39 (6)	33.3
60–70	2/6	33.3	1/7	14.2
Prior chemotherapy				
1 regimen	—		6/25 (3)	24
⩾ 2 regimens	7/12 (1)	58.3	8/21 (3)	38

*No. of responders = complete + partial. Values in parentheses = No. of patients achieving complete remission.

†Median response duration = 3 mos (range, 2–13).

‡Median response duration = 5 mos (range, 1–26+).

response was documented in five of ten patients with nodular sclerosis histology and in two of two with mixed cellularity histology, while in non-Hodgkin's lymphoma, complete plus partial remission was observed in two of six patients with follicular lymphoma, in seven of 27 with diffuse, and in five of 13 with mixed lymphocytic-histiocytic pattern of growth, respectively. The breakdown of results between *m*-AMSA and *m*-AMSA lactate failed to detect important differences in terms of incidence and duration of response (table 3).

Table 4 presents the side effects encountered during therapy with the amsacrines. However, the degree of hematologic toxicity was not increased by increasing the dose of both drugs, and *m*-AMSA lactate induced less severe thrombocytopenia than *m*-AMSA. For both drugs, the nadir for wbc and platelet counts ranged from Days 10 to 14. A fatality related to treatment occurred in the *m*-AMSA group in a 32-year-old patient with diffuse histiocytic lymphoma extensively treated with prior chemotherapy. Following the first course with 90 mg/m^2, the wbc count unexpectedly fell to 200/mm^3 and the platelet count fell to 19,000/mm^3. The patient died in almost complete remission because of pneumonia; her bone marrow had probably not completely recovered from previous lomustine administration. Local venous irritation was observed in the first

TABLE 3.—Results related to *m*-AMSA or *m*-AMSA lactate*

	m-AMSA		*m*-AMSA lactate	
	Hodgkin's disease	Non-Hodgkin's lymphoma	Hodgkin's disease	Non-Hodgkin's lymphoma
Entered	3/6	6/28	4/8	8/28
Evaluable	3/6	6/23 (3)	4/6 (1)	8/23 (3)
Dose (mg/m^2)				
90–120	3/5	4/16 (2)		
70	—	1/3		
25–30 for 3 days	0/1	1/4 (1)		
Disease extension				
Nodal	1/1	3/10 (3)	2/2 (1)	6/9 (1)
Extranodal	2/5	3/13	2/4	2/14 (2)
Response duration (mos)				
Median	2	4	4.5	2
Range	2–13	1–26+	2–12	1–6+

*Unless specified otherwise, values = No. of responders (complete + partial)/total. Values in parentheses = No. of patients achieving complete remission.

TABLE 4.—Comparative toxic effects in evaluable patients*

Toxic effect	*m*-AMSA (N = 29)	*m*-AMSA lactate (N = 29)
Vomiting	54	45
Fever/chills	4	3
Stomatitis	0	3
Alopecia	7	14
Infections	3†	0
Phlebitis	0	14
Leukopenia (cells/mm^3)		
< 3000	43	55
< 1000	21	3
Thrombocytopenia (cells/mm^3)		
< 100,000	29	31
< 75,000	21	14

*Values = % of patients.
†One patient with fatal pneumonia.

series of patients treated with *m*-AMSA lactate. This immediate side effect disappeared by injecting an iv saline infusion for a few minutes following drug administration. No patient had renal and/or liver abnormalities related to treatment with amsacrines.

No important cardiac toxic effects were documented in the 13 patients given *m*-AMSA lactate and evaluated with systolic time intervals. Transient ECG changes were represented by flattened T waves, sporadic atrial premature beats, and ventricular premature beats in 31% of the patients.

DISCUSSION

The results of our study demonstrated that amsacrines in advanced refractory malignant lymphomas were effective, but caused moderate toxicity. In fully evaluable patients, amsacrines were also able to induce complete remissions, and the objective response was inversely related to performance status and presence of extranodal disease. With the dose schedules utilized, the remission rate was higher in patients with Hodgkin's disease (58.3%) than in patients with non-Hodgkin's lymphoma (30.4%). Furthermore, the therapeutic activity was comparable between *m*-AMSA and *m*-AMSA lactate.

From the therapeutic point of view, our findings appear more favorable than most of those reported in other studies (10,13,16–18), and are more in agreement with the recent results of Case (19), who reported complete plus partial remissions in 45% of 22 patients with non-Hodgkin's lymphoma.

The observed difference is probably due in large part to patient selection. In fact, disease extent, low performance status, and poor bone marrow reserve due to several prior drug treatments may well have prevented the attainment of objective response in most cases. However, the possibility that the number of relapses before treatment with amsacrines could affect the response rate, as observed by Cabanillas et al (18), was not confirmed by our experience.

This study confirms that bone marrow toxicity represented the major dose-limiting factor. Although various dose schedules of amsacrines were tried in the past (10,11,13,20), the 3-week schedule utilizing *m*-AMSA lactate at the single dose of 225 mg/m^2 appears effective and devoid of major side effects (21). Our experience failed to confirm that amsacrines may produce acute or chronic cardiac toxicity in a few patients, as reported by other investigators (19,21,22). Serial systolic time intervals recorded in 13 patients showed no laboratory signs of myocardial dysfunction. Only a few aspecific and transient ECG changes were recognized.

In conclusion, our favorable results with *m*-AMSA and *m*-AMSA lactate in refractory malignant lymphomas, particularly in Hodgkin's disease, suggest that amsacrine is worthy of further trials in these hematologic malignancies. As recently reported in a limited series of 13 patients by Berkman et al (23), this agent could probably be effectively incorporated into polydrug therapy as a salvage regimen in malignant lymphomas, and could probably also be incorporated into the multistep strategy for primary therapy in high-risk patients.

REFERENCES

1. CAIN BF, ATWELL GJ, and SEELEY RN. Potential antitumor agents. 12.9-Anilinoacridines. J Med Chem 15:611–615, 1972.
2. CAIN BF, and ATWELL GJ. The experimental antitumor properties of three congeners of the acridyl-methane sulfonamide (AMSA) series. Eur J Cancer 10:539–549, 1974.
3. ROZENCWEIG M, VON HOFF DD, CYSYK RL, ET AL. *m*-AMSA and PALA: two new agents in cancer chemotherapy. Cancer Chemother Pharmacol 3:135–141, 1979.
4. LEGHA SS, GUTTERMAN JU, HALL SW, ET AL. Phase I clinical investigation of 4′-(9-acridinylamino) methanesulfon-*m*-anisidide (NSC-249992), a new acridine derivative. Cancer Res 38:3712–3716, 1978.
5. HALL SW, BENJAMIN RS, LEGHA SS, ET AL. Clinical pharmacokinetics of the new antitumor agent AMSA. Proc Am Assoc Cancer Res and ASCO 20:175, 1979.
6. LEGHA SS, LATREILLE J, MCCREDIE KB, ET AL. Neurologic and cardiac rhythm abnormalities associated with 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (AMSA) therapy. Cancer Treat Rep 63:2001–2003, 1979.
7. VON HOFF DD, ELSON D, POLK G, ET AL. Acute ventricular fibrillation and death during infusion of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (AMSA). Cancer Treat Rep 64:356–358, 1980.
8. STEINHERZ LJ, STEINHERZ PG, MANGIACASALE D, ET AL. Cardiac abnormalities after AMSA administration. Cancer Treat Rep 66:483–488, 1982.
9. ARLIN ZA. Current status of amsacrine (AMSA) combination chemotherapy programs in acute leukemia. Cancer Treat Rep 67:967–970, 1983.
10. WEICK JK, JONES SE, and RYAN DH. Phase II study of amsacrine (*m*-AMSA) in advanced lymphomas: a Southwest Oncology Group study. Cancer Treat Rep 67:489–492, 1983.

11. AHMANN FR, MEYSKENS FL, JR, JONES SE, ET AL. Phase II evaluation of amsacrine (*m*-AMSA) in solid tumors, myeloma and lymphoma: a University of Arizona and Southwest Oncology Group study. Cancer Treat Rep 67:697-700, 1983.
12. CABANILLAS F, LEGHA SS, BODEY GP, ET AL. Initial experience with *m*-AMSA as single agent treatment against malignant lymphoproliferative disorders. Blood 57:614-616, 1981.
13. GOLDSMITH MA, BHARDWAY S, OHNUMA T, ET AL. Phase I study of *m*-AMSA in patients with solid tumors and leukemias. Cancer Clin Trials 3:197-202, 1980.
14. WEISSLER AM, HARRIS WS, and SCHOENFELD CD. Bedside techniques for the evaluation of ventricular function in man. Am J Cardiol 23:577-583, 1969.
15. WORLD HEALTH ORGANIZATION. Handbook for Reporting Results of Cancer Treatment. WHO Offset Publ No. 48. Geneva, WHO, 1979.
16. WARRELL RP, JR, STRAUS DJ, and YOUNG CW. Phase II trial of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (AMSA) in the treatment of advanced non-Hodgkin's lymphoma. Cancer Treat Rep 64:1157-1158, 1980.
17. CABANILLAS F, HAGEMEISTER FB, MCLAUGHLIN P, ET AL. Long-term outcome of complete responders (CR) to salvage chemotherapy for recurrent or refractory lymphoma. Proc ASCO 2:213, 1983.
18. CABANILLAS F, HAGEMEISTER FB, SALVADOR P, ET AL. Factors predicting for complete remission (CR) in patients (PTS) with recurrent lymphoma treated with salvage combination chemotherapy. Proc ASCO 1:167, 1982.
19. CASE DC. *m*-AMSA: phase II trial in advanced lymphoma and leukemia. Am J Clin Oncol 7:357-360, 1984.
20. BRAMWELL VH, HOLDENER EE, SIEGENTHALER P, ET AL. Phase II study of amsacrine in refractory lymphomas. A Report of the EORTC Early Clinical Trials Group, 1984.
21. TAN CTC, HANCOCK C, STEINHERZ PG, ET AL. Phase II study of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (NSC-249992) in children with acute leukemia and lymphoma. Cancer Res 42:1579-1581, 1982.
22. WEISS RB, MOQUIN D, ADAMS JD, ET AL. Electrocardiogram abnormalities induced by amsacrine. Cancer Chemother Pharmacol 10:133-134, 1983.
23. BERKMAN A, LENHARD R, TRUMP D, ET AL. Intensive chemotherapy with cyclophosphamide (C), *m*-AMSA (AM) and VP16 (CAMP) as treatment for refractory and relapsing non-Hodgkin's lymphoma (NHL). Proc ASCO 2:211, 1983.

Rate of M-Component Changes and Plasma Cell Labeling Index in 25 Patients With Multiple Myeloma Treated With Peptichemio[1,2]

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Twenty-five consecutive patients with previously untreated multiple myeloma were studied for bone marrow plasma cell labeling index, response to peptichemio induction therapy, and rate of M-component (MC) changes during the course of the disease. They received intermittent melphalan or cyclophosphamide as maintenance therapy and peptichemio associated with vincristine at first relapse. The response rate (76%) was independent of clinical stage, evaluated according to Merlini et al and to Durie and Salmon. Among responsive patients, rapid responders (half-life of MC decrease < 47 days for IgA and IgG and < 29 days for light chain myelomas) had a bone marrow plasma cell labeling index significantly higher ($P < 0.01$) than that of slow responders. Rapid responders had a median survival of 15 months, while slow responders had a median survival of 42 months ($P < 0.05$). The difference in survival between the two groups was accounted for mainly by the difference in duration of first response and the different rates of MC increase following it. Both of these parameters were directly related to the half-life of MC decrease at response in IgA and IgG myelomas. The duration of second response and the half-life of MC increase following it were shorter than the duration of first response and than the half-life of MC increase at first relapse. [Cancer Treat Rep 69:971–975, 1985]

Other than response to therapy, a number of clinical and biological factors influence survival of myeloma patients. Bad prognostic significance of renal failure, severe anemia, and high bone marrow plasma cell (BMPC) percentage is taken into consideration by recently devised clinical staging systems (1,2). Among biological factors, high proliferative activity of BMPC (as determined by in vitro tritiated thymidine labeling index, ^{3}H-TdR LI) at diagnosis is a forerunner of reduced survival (3,4).

In 1969, Hobbs (5) also reported that patients responsive to melphalan (MPH) or to cyclophosphamide (CTX) within 3 months had a shorter survival than those who responded more slowly. This observation was then confirmed (4,6). However, little attempt has been made to relate the degree of proliferative activity to the time needed for response and to examine the myeloma's clinical course in rapid and slow responsive patients.

In this study, 25 patients who had BMPC LI determination prior to therapy were followed for the changes in serum or urine M-component (MC) level following peptichemio (PTC) induction therapy and subsequently during the course of the myeloma.

MATERIALS AND METHODS

Twenty-five consecutive patients (14 male and 11 female; median age, 59 years) with previously untreated multiple myeloma (7) received PTC as the first induction therapy. Thirteen of these patients had IgG myeloma, six had IgA myeloma, and six had light chain (LC) myeloma. In patients with LC disease, Bence Jones (BJ) protein excretion was > 1.5 g/day (range, 1.5–60.8).

Treatment

For response induction, PTC (Istituto Sieroterapico Milanese, Milan, Italy) was administered cyclically at a dose of 0.4–0.8 mg/kg/48 hours repeated three to eight times (8). Each patient received two or three courses of treatment at 10–15-day intervals. Prednisone was given during the treatment courses and then rapidly tapered.

[1]Received Mar 20, 1984; revised Jan 30, 1985; accepted Feb 12, 1985.

[2]Supported by Consiglio Nazionale delle Ricerche-Roma-Progetto Finalizzato Oncologia, grant 8400761.

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As soon as response was achieved, maintenance therapy with intermittent MPH or CTX was started. First relapse was treated with the combination of PTC and vincristine (VCR). VCR (0.025 mg/kg) was administered on the first day of each PTC course (9). Resistant patients were treated with MPH or with different combination chemotherapy protocols (10).

Clinical Evaluation

The extent of myeloma at diagnosis was evaluated by determining clinical stage (2) and tumor mass (TM) (11). Prior to therapy and serially 5–40 days after beginning it, the following parameters were determined: serum and urine concentration of MC (as the scanning percentage of total serum and urine proteins), BMPC percentage, hemoglobin, serum calcium, creatinine, and skeletal lytic lesions. The criteria for response were chosen according to the Chronic Leukemia and Myeloma Task Force (7). Patients were considered as responders when a decrease of $> 50\%$ in serum MC concentration or in urinary MC excretion was documented.

Duration of response is defined as the interval between the time when the minimal MC level at response was reached and the time when this level was increased by 50%. At this time, the diagnosis of relapse was made. Duration of stable response is the interval between time when the minimal MC level at response was reached and time when this level was increased by 25%. Survival is calculated from starting treatment.

Rate of MC Changes

The rate of MC changes was evaluated as MC half-life, ie, time with which the MC level increased or decreased by 50%. The half-life of MC changes was calculated from MC production rate (product of serum concentration and fractional catabolic rate) for IgG myelomas, from MC concentration for IgA myelomas, and from MC urinary excretion for LC myelomas. To determine the half-life of MC decrease at response, MC levels were evaluated before therapy and serially thereafter until the minimal level at response was reached. To determine the half-life of MC increase at relapse, the patients were evaluated starting from the time when MC was increased by 25% with respect to the minimal value at response to the time when diagnosis of relapse was made (increase of $\geq 50\%$).

Labeling Index

The ^{3}H-TdR LI of BMPC was determined in vitro prior to therapy. The method is described elsewhere (12). Since the background was 4 grains/100 μm^2, cells with ≥ 5 grains were considered as labeled.

Statistical Analysis

Distribution of LI among patients grouped according to clinical stage, TM, and half-life of MC decrease following first induction therapy was studied using the Wilcoxon test. The Fisher's exact test was employed to examine differences in response to treatment. Differences in survival were analyzed using (*a*) the log-rank test (13) and (*b*) the procedure described by Hankey and Myers (14), which takes into account differences in distribution between two groups with respect to factors (stage, half-life of MC decrease, and BMPC LI) thought to be associated with survival.

RESULTS

The results are shown in table 1 and in figures 1–3.

Response and Survival According to Clinical Stage and TM

For the whole series, the overall response rate was 76% (19 responses among 25 patients) and the median survival was 27 months (range, 1–113+; 30+ months for responders and 5.5 months for nonresponders). Response rate was not affected by stage or TM (table 1). Median survival was longer in stage I than in stage II and III

TABLE 1.—Response rate, survival, and half-life of MC decrease in responsive patients and BMPC LI by stage (ref 2) and TM (ref 11)*

Parameter	Stage				TM		
	I	II	III	*P* value	Intermediate	High	*P* value
Response	5/7†	4/7†	10/11†	> 0.05	9/12‡	10/13‡	> 0.05
Median survival (mos)	57	27	18	< 0.05	42	24	< 0.05
Half-life of MC decrease (days)							
Serum spike multiple myeloma	45	48	56	> 0.05	35	60	> 0.05
LC multiple myeloma	—	—	29	—	—	29	—
BMPC LI (%)	2.10	3.15	2.00	> 0.05	2.08	2.08	> 0.05

* Intermediate TM $< 1.2 \times 10^{12}$ cells; high TM $> 1.2 \times 10^{12}$ cells.
†No. of patients responding/total No. with stage indicated.
‡No. of patients responding/total No. with TM indicated.

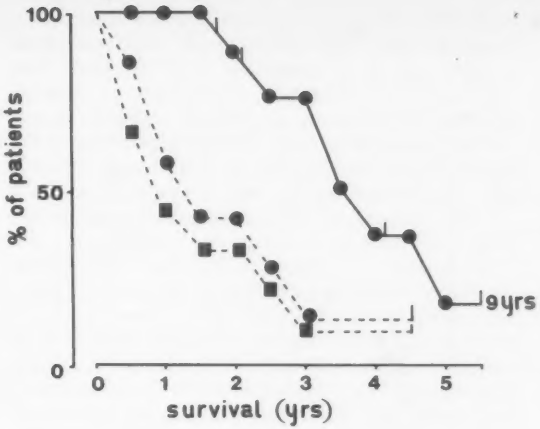

FIGURE 1.—Survival of responsive myeloma patients according to half-life of MC decrease following PTC induction therapy. ●——● = slow responders (half-life > 47 days for serum spike and > 29 days for LC myelomas). ●----● = rapid responders (half-life < 45 days for serum spike and < 29 days for LC myelomas). ■----■ = rapid responders after excluding 2 patients who died before maximum half-life of MC decrease observed among all responders.

myelomas and it was longer in patients with intermediate TM than in those with high TM.

Survival According to the Half-Life of MC Decrease

Among PTC-responsive patients, the median half-life of MC decrease was 47 days (range, 18–136) in the 13 with serum spike myeloma and 29 days (range, 5–296) in the six with LC myeloma. No correlation was seen with stage or TM (table 1).

To assess whether the half-life of MC decrease influences survival, responsive patients were divided into two groups having a half-life less than the median (rapid responders, nine patients) or greater than or equal to the median (slow responders, ten patients). Survival curves were constructed either including all responders or excluding those who died within a time shorter than the maximum half-life of MC decrease we observed (136 days for serum spike and 296 days for LC myelomas). This second survival analysis was performed to avoid the bias of patient selection, since slow responders survive, by definition, at least as long as their time to respond.

Figure 1 shows that the median survival was 42 months (range, 22+ to 113+) for slow responders, 10 months (range, 2–55+) for all rapid responders ($P < 0.05$), and 15 months (range, 5–55+) for rapid responders, after excluding two rapidly dying patients according to the above considerations ($P < 0.05$). These figures remain unchanged using either the log-rank test or the method of Hankey and Myers (14).

Response and Survival According to LI

Median BMPC LI was 2.08% (mean ± SD = 3.10 ± 2.96; range = 0.01–16.06). LI values were uniformly distributed among clinical stages and TM groups (table 1). The three highest values (6.03%, 6.53%, and 16.06%), however, were found in patients with both stage III and high TM myelomas. Patients with LI > 2.08% had a median survival of 9 months (range, 1–57), and those with LI < 2.08% had a median survival of 30 months (range, 12–113+) ($P = 0.03$).

Median LI was 1.85% (range, 0.01%–6.53%) in responsive patients and 3.38% (range, 2.10%–16.06%) in unresponsive patients ($P < 0.05$). Among responsive patients, those with LI > 1.85% had a median survival of 18 months and those with LI < 1.85% had a median survival of 40 months ($P > 0.05$).

LI and Half-Life of MC Decrease at First Response

Rapid responders had a median LI of 2.73% (range, 0.50%–6.53%) and slow responders had a median LI of 1.45% (range, 0.01%–2.16%) ($P < 0.01$).

Rate of MC Changes Following the First Response

A portion of the 19 responsive patients had MC determinations frequent enough to follow the rate of MC level changes after first treatment.

In 12 patients (seven slow and five rapid responders) with IgA or IgG myeloma, the duration of the overall response was directly related ($P < 0.02$) to the half-life of MC decrease at response (fig 2). This correlation was not found among five LC myelomas. Only one of the rapid responders (a patient with LC disease) experienced a stable response of 6.5 months, while all patients who responded slowly had stable response of 3.2–98+ months.

In ten patients with either serum spike or LC myeloma who relapsed during maintenance therapy, the half-life of MC increase was directly related ($P < 0.01$) to the half-life of MC decrease following initial induction therapy.

In eight patients responsive to PTC-VCR at relapse, the half-lives of MC decrease were similar at first and at second induction therapy (fig 3A).

The duration of second response was short in all patients studied except one (with LC disease) and was less than the duration of first response (fig 3B).

In six patients, the half-life of MC increase after the second response was shorter than the half-life of MC increase at first relapse (fig 2C).

DISCUSSION

The aim of this study was to ascertain whether a correlation exists between two parameters, BMPC LI and rate of response to treatment, recognized to be of prognostic

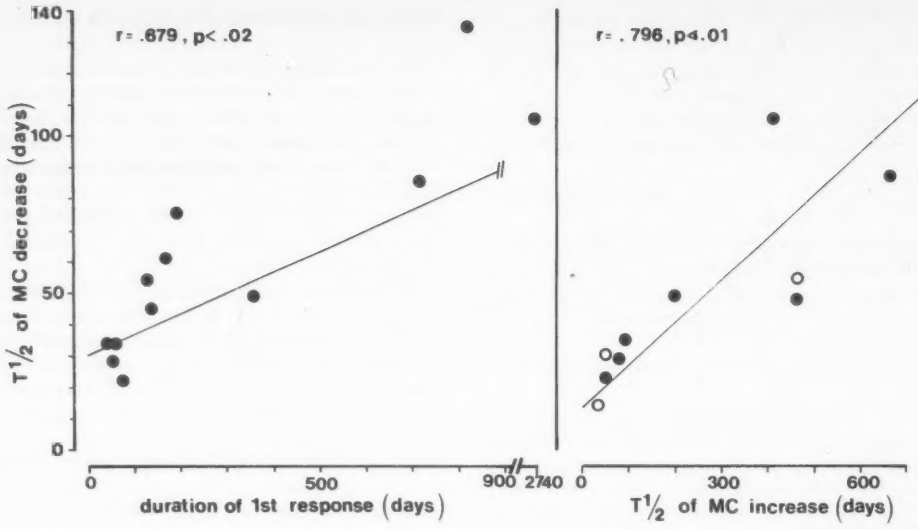

FIGURE 2.—Correlation between half-life of MC decrease following PTC induction therapy and duration of 1st response (left panel) and half-life of MC increase thereafter (right panel).

significance in responsive myelomas. The number of patients studied was quite small. However, suggestion can be made that BMPC LI and rate of response to PTC (measured as half-life of MC decrease) are linked to each other. Patients who promptly responded to PTC had a BMPC LI significantly higher (median, 2.73%) than those who responded more slowly (median, 1.45%). Survival was shorter in rapid than in slow responders.

Patients with high S-phase values (TM times LI) and rapid tumor regression but short survival were described by Durie et al (4) after MPH or CTX treatment. The reasons for this behavior were not reported. In PTC-sensitive patients, response is usually more rapid (8) than in MPH-sensitive patients. Rate of response can be determined with accuracy, since most patients are treated in the hospital for early induction and serial MC determinations are needed to decide when maintenance therapy must be instituted. The shorter survival in rapid than in slow responders seems to be related, at least in serum spike myelomas, to the different duration of first response and the different speed of relapse. Rapid responders, whose LI was often high, had short-lasting first remissions, with rapid onset of relapse during maintenance therapy. On the contrary, the duration of first response was more prolonged and the increase in MC level following it was slower in slow responders with a low LI. In both rapid and slow responders, the course of myeloma after second induction was always accelerated with respect to the initial phase (from first induction to first relapse), as already observed by Bergsagel and Pruzansky (15). As stated above, due to the small number of patients studied, firm conclusions and confirmatory studies are needed.

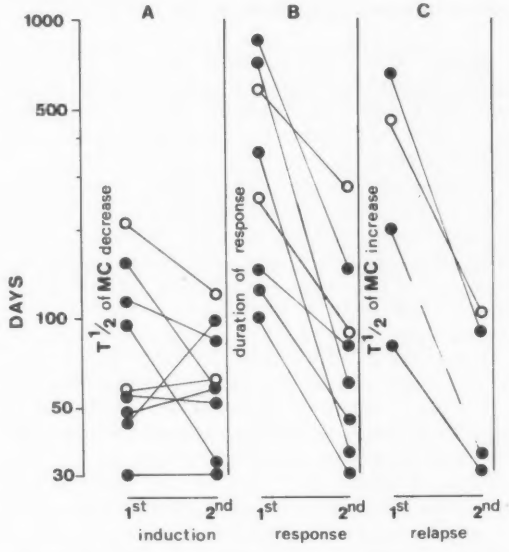

FIGURE 3.—Rates of MC changes and durations of response in individual patients with multiple myeloma during the course of the disease. A—half-life of MC decrease at 1st and 2nd induction therapy. B—duration of 1st and 2nd response. C—half-life of MC increase at 1st and 2nd relapse (● = patients with IgG or IgA myeloma; ○ = patients with LC myeloma).

To explain the accelerated course of the myeloma in rapid responders, the following points must be considered. Intrinsic drug sensitivity of plasma cells is increased by their high LI, which leads to a rapid TM reduction. Resistant plasma cells, whose burden is already low in rapid responders (16), are thus easily recruited into the cell cycle (17-19). These clones are probably different from the sensitive ones and are selected from both PTC induction therapy and MPH maintenance therapy. The quite frequent occurrence of markedly aneuploid plasma cell clones with high LI that we observed in rapidly relapsing patients (20) supports this interpretation. These events need to be further investigated using more accurate cytokinetic techniques, such as the estimation of the growth fraction (21) and the combined determination of DNA content, LI, and DNA synthesis time (22) of individual myeloma cells. This last method will allow one to define the proliferative characteristics of the different plasma cell clones.

REFERENCES

1. DURIE BGM, and SALMON SE. A clinical staging system for multiple myeloma. Cancer 35:842-854, 1975.
2. MERLINI GP, WALDENSTROM JG, and JAYAKAR SD. A new improved clinical staging system for multiple myeloma based on the analysis of 123 treated patients. Blood 55:1011-1019, 1980.
3. LATREILLE J, BARLOGIE B, JOHNSTON D, ET AL. Ploidy and proliferative characteristics in monoclonal gammopathies. Blood 59:43-51, 1982.
4. DURIE BGM, SALMON SE, and MOON TE. Pretreatment tumor mass, cell kinetics and prognosis in multiple myeloma. Blood 55:364-372, 1980.
5. HOBBS JR. Growth rates and response to treatment in human myelomatosis. Br J Haematol 16:607-617, 1969.
6. HANSEN OP, JENSSEN B, and VIDEBAEK A. Prognosis of myelomatosis on treatment with prednisone and cytostatics. Scand J Haematol 10:282-290, 1973.
7. CHRONIC LEUKEMIA AND MYELOMA TASK FORCE. Proposed guidelines for protocol studies. II. Plasma cell myeloma. Cancer Chemother Rep Part 3, vol 1(1):17-39, 1968.
8. MERLINI GP, GOBBI PG, RICCARDI A, ET AL. Peptichemio induction therapy in myelomatosis. Cancer Chemother Pharmacol 8:9-16, 1982.
9. MERLINI GP, RICCARDI A, RICCARDI PG, ET AL. Peptichemio, vincristine and prednisone induction treatment in multiple myeloma. *In* Proceedings of the 13th International Chemotherapy Congress. Wien, Austria, Aug 28-Sept 2, 1983, Part 250, pp 22-25.
10. WINTROBE M. Multiple myeloma. *In* Clinical Hematology, 8th ed. Philadelphia, Lea and Febiger, 1981, pp 1739-1760.
11. SALMON SE, and WAMPLER SB. Multiple myeloma: quantitative staging and assessment of response with a programmable pocket calculator. Blood 49:379-389, 1977.
12. RICCARDI A, MAZZINI G, MONTECUCCO CM, ET AL. Sequential vincristine arabinosylcytosine and adriamycin in acute leukemia: cytologic and cytokinetic studies. Cytometry 3:104-109, 1982.
13. PETRO R, PIKE MC, ARMITAGE P, ET AL. Design and analysis of randomized trials which require prolonged observations of each patient. II. Analysis and examples. Br J Cancer 35:1-39, 1977.
14. HANKEY BF, and MYERS MH. Evaluating differences in treatment between two groups of patients. J Chronic Dis 24:523-532, 1971.
15. BERGSAGEL DE, and PRUZANSKY W. Treatment of plasma cell myeloma with cytotoxic agents. Arch Intern Med 135:172-176, 1975.
16. HOKASON JA, BROWN BW, THOMPSON JR, ET AL. Tumor growth patterns in multiple myeloma. Cancer 39:1077-1084, 1977.
17. PILERI A, BARNENGO MG, BOCCADORO M, ET AL. Early recruitment in the human myeloma cell population after cytostatic treatment. Haematologia 61:184-193, 1976.
18. KARP JE, BURKE PJ, and HUMPHREY RL. Induction of serum stimulation and plasma cell proliferation during chemotherapy of multiple myeloma. Blood 49:925-934, 1977.
19. RICCARDI A, MARTINOTTI A, and PERUGINI S. Cytokinetic changes in two cases of plasma cell leukemia treated with a multipeptide derivative of *m*-{di(2-chloroethyl)amino}-L-phenylalanine (peptichemio). Eur J Cancer 14:1099-1107, 1978.
20. MONTECUCCO CM, RICCARDI A, MERLINI GP, ET AL. Plasma cell DNA content in multiple myeloma and related paraproteinemic disorders. Relationship with clinical and cytokinetic features. Eur J Cancer Clin Oncol 20:81-90, 1984.
21. DREWINKO B, ALEXANIAN R, BOYER H, ET AL. The growth fraction of human myeloma cells. Blood 57:333-338, 1982.
22. GIORDANO P, MAZZINI G, RICCARDI A, ET AL. A simple procedure for DNA cytofluorometry simultaneously with autoradiographic grain count. *In* Proceedings of the Combined International Conference on Analytical Cytology and VIth International Symposium on Flow Cytometry. Elmau, West Germany, 1982, p 75.

Endocrine Effects of Medroxyprogesterone Acetate: Relation Between Plasma Levels and Suppression of Adrenal Steroids in Patients With Breast Cancer[1]

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The elimination of adrenal steroids by adrenalectomy or medical treatment has been one of the mainstays in the palliative treatment of patients with disseminated breast cancer. Recently, the effects of progestins on adrenal function has become the subject of renewed interest. To find a relation between plasma levels of medroxyprogesterone acetate (MPA) and adrenal suppression in breast cancer patients, we compared a group of 41 postmenopausal patients with disseminated or inoperable breast cancer, treated with oral MPA, with a control group of 28 postmenopausal breast cancer patients without treatment. To obtain MPA plasma levels in the lower range, 13 patients initially received 300 mg of MPA daily (Group 1), six of whom were subsequently treated with 600 mg (Group 2). Group 3 comprised 37 patients treated with 900 mg of MPA. All of the parameters studied varied widely. Only in Group 3 patients were the MPA levels higher and the levels of cortisol, androstenedione, DHAS, and estrone lower than those in Group 1 patients. All steroid levels in Group 3 were significantly below control values. Considering all data, a negative but weak correlation was found between MPA levels and cortisol (R = –0.55), androstenedione (R = –0.58), and dehydro-epi-androsterone sulfate (R = –0.40), but not for MPA and estrone, indicating better adrenal suppression by higher MPA dosage and plasma levels. However, suppressed cortisol concentrations did not predict sufficient MPA dosage in all patients because low cortisol was not always associated with high MPA levels. A positive relation existed between the levels of androstenedione and estrone, which shows that suppression of these adrenal androgens may result in suppression of plasma estrone. This may be of additional therapeutic benefit in breast cancer patients. [Cancer Treat Rep 69:977–983, 1985]

Recently there has been extensive debate about the potentially greater effectiveness of high-dose progestins in the treatment of patients with disseminated breast cancer. Although earlier investigators described rather low remission rates (1–3), a recent review mentions remission percentages of 30%–40% in unselected patients (4). Minimal effective medroxyprogesterone acetate (MPA) plasma levels have been stated, though a wide variation exists for individual patients (5). Recently, we found only partial adrenal suppression during daily use of 900 mg of MPA orally (8), although complete suppression of adrenal function during higher MPA dosages has been described by some investigators (6,7).

It has been known for a long time that the elimination of adrenal steroids by surgical adrenalectomy or suppression by drugs such as aminoglutethimide may inhibit the growth of hormone-sensitive breast cancer cells, probably by suppression of adrenal androgens, that serve as the source of the main postmenopausal estrogen, estrone.

We have tried to establish a relation between the plasma levels of MPA during the use of different dosages as determined by radioimmunoassay (RIA) and the suppression of adrenal steroids, which might be used as a biologic parameter of the presence of a sufficient MPA plasma concentration. Indeed, part of the

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[4]We thank Mr. V. Tamassia, Farmitalia Carlo Erba, for determination of the medroxyprogesterone acetate levels; Mr. J. Smeets, Research Director Benelux Farmitalia, for his support; Prof. Dr. J. H. H. Thijssen, Endrocrinologic Laboratory of the University of Utrecht, for determination of estrone levels; and Mrs. W. J. A. Bruins-van der Weij for her excellent secretarial assistance.

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mechanism of action of MPA may rest on suppression of the adrenal androgens, which serve as precursors of plasma estrone.

PATIENTS AND METHODS

In a multicenter, randomized trial, the effects of three doses of 300 mg of MPA and two doses of 20 mg of tamoxifen as the initial treatment in postmenopausal patients with measurable or evaluable advanced breast cancer were compared. In the two main participating centers (University Hospital of Groningen and Deaconess Hospital of Leeuwarden), it was decided to study the effects of MPA on the plasma levels of the adrenal steroids cortisol, dehydro-epi-androsterone sulfate (DHAS), androstenedione, and estrone in all 28 consecutive patients entering the MPA arm and to compare the results with the pretreatment values obtained in all 28 consecutive patients entering the tamoxifen arm.

To obtain plasma levels of the above-mentioned steroids during treatment with MPA at a lower dosage, a small number of patients were treated with escalating dosages, since we wanted to demonstrate a correlation between the MPA levels and these steroids. These patients did not have measurable or evaluable disease, but were otherwise comparable to the trial patients. Thirteen patients were first given three doses of 100 mg of MPA orally (Group 1). Three patients were not eligible for dose escalation because of tumor progression, six subsequently received three doses of 200 mg of MPA (Group 2), and four received three doses of 300 mg of MPA. Five patients from Group 2 also received three doses of 300 mg of MPA. Therefore, Group 3 comprised the 28 patients initially treated with three doses of 300 mg together with five patients from Group 2 and four from Group 1, for a total number of 37 patients. Thus, 41 postmenopausal patients with advanced breast cancer (mean age, 61 yrs; range, 53–74) were studied during treatment with MPA, and 28 patients (mean age, 63 yrs; range, 52–75) from the tamoxifen arm were studied before treatment was given.

Because MPA levels in plasma rise gradually, patients had to be treated for at least 6 weeks before plasma samples were taken. All samples for MPA and steroid determinations were collected between 9:00 AM and 10:00 AM, while the last MPA dose had been taken at 11:00 PM. Patients and controls were free of endrocrine disturbances, severe malaise, or systemic symptoms and had a Karnofsky score > 70.

Plasma MPA levels were determined by RIA according to Shrimankar et al (9). Determination of cortisol, DHAS, and androstenedione has been described earlier (10). Plasma estrone was determined by RIA (11).

Statistical comparisons were made by the Wilcoxon test and rank correlations were calculated according to Spearman. When the correlations for single-dose levels were calculated in all 41 patients receiving MPA, we used the levels of the 28 patients in Group 3 that were studied once, of all patients in Group 2, and of the remaining seven patients in Group 1.

RESULTS

Median MPA levels in Group 3 were the highest and different from those reached in Group 1. In 14 of the 37 patients the levels were still < 100 ng/ml, despite treatment for at least 6 weeks before the samples were taken (represented as log MPA in fig 1).

The difference in plasma steroid levels between Group 1 versus Group 2 or Group 2 versus Group 3 did not reach statistical significance (table 1). The levels of all steroids in Group 3 were lower than those in Group 1 and markedly below control values, while in Group 1 only DHAS and androstenedione fell below the normal range (table 1). A negative correlation was found between individual MPA levels and the plasma cortisol concentration in all samples ($R = -0.55$, $P < 0.01$) (fig 2).

DHAS concentration exhibited a negative correlation with individual MPA levels ($R = -0.40$, $P < 0.01$) (fig 3), as did androstenedione levels ($R = -0.58$, $P < 0.01$) (fig 4). These correlations are influenced by the fact that some patients were studied on more than one dose

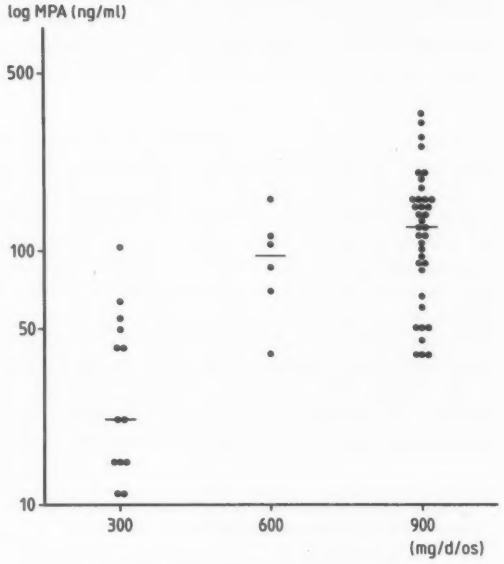

FIGURE 1.—Plasma MPA concentrations during the use of 300, 600, and 900 mg orally daily.

TABLE 1.—Plasma levels of MPA, cortisol, DHAS, androstenedione, and estrone in Groups 1-3 and in untreated breast cancer controls

Steroid	Group 1		Group 2		Group 3		Control level
	Level	P vs Group 2	Level	P vs Group 3	Level	P vs Group 4	
MPA (ng/ml)							
Median	23	NS	98	NS	126	< 0.01	–
Range	4-103		41-177		38-364		–
Cortisol (nmols/L)							
Median	455	NS	125	NS	70*	< 0.01	395
Range	115-1105		30-640		10-465		155-785
DHAS (μmols/L)							
Median	1300†	NS	195	NS	485*	< 0.01	2440
Range	115-4590		40-1440		55-1220		1015-6340
Androstenedione (nmols/L)							
Median	2.85*	NS	1.18	NS	1.16*	< 0.01	3.75
Range	1.14-4.43		0.72-2.28		0.56-3.10		1.23-9.81
Estrone (pmols/L)							
Median	105	NS	85	NS	70*	< 0.02	126
Range	75-202		54-115		40-130		22-252

*$P < 0.01$ vs control.
†$P < 0.05$ vs control.

level. Thus, when all patients were studied only on one dose level (seven receiving 300, six receiving 600, and 28 receiving 900 mg of MPA daily), the correlations were more extreme, ie, R = -0.42 ($P < 0.02$) for cortisol, R = -0.36 ($P < 0.05$) for androstenedione, but no significant correlation could be found for DHAS. On the other hand, studying only those patients who were sampled two or three times (five patients receiving 300, 600, and 900 mg, five on two dose levels), the correlation between MPA and cortisol was R = -0.79 ($P < 0.001$), androstenedione was R = -0.65 ($P < 0.01$), and DHAS was R = -0.58 ($P < 0.01$).

Estrone levels in Group 3 were lower than those in control patients and those in Group 1. A positive correlation was found between the levels of androstenedione and estrone in all patients together (R = 0.73, $P < 0.01$) (fig 5).

DISCUSSION

Although the necessity of sufficient MPA dosages to reach adequate plasma levels has been stressed repeatedly (5,12), there still is considerable debate on the difference in clinical effect of high versus low MPA dosage. Two reviews of older studies using 100-400 mg of oral progestins daily showed an overall response rate of 17% compared with 40% in more recent studies using 500-2000 mg/day im (4,13). The effect of dose levels up to 2000 mg/day, however, was not superior to 500 mg/day im. During the oral use of 300 versus 900 mg of MPA daily, remission rates of 16% and 24%, respectively, were reported, a difference that did not reach statistical significance (14). Perhaps this was due to the fact that MPA in these patients was not the primary hormonal treatment. No attempts have been made, however, to compare the plasma MPA levels reached by MPA responders with those of nonresponders.

In the present study, plasma MPA concentrations in patients treated with 300 mg of MPA were lower than in the group treated with 900 mg. In all groups, however, a large variation between individual levels was found, which is in accordance with other studies (7,15). There are a number of factors that may account for these findings.

First, there is considerable cross-reaction by MPA metabolites in the RIA procedure, and lower but variable levels have been reported using gas chromatography (16) or high-performance liquid chromatography (17). With all methods, however, remarkable interindividual variations have been described (9,15,18). MPA resorption may be erratic (19,20), depending on its galenic form (21) or metabolism by intestinal flora (22). Furthermore, there seems to be considerable variation in body distribution and metabolite clearance of progestins between individuals (23) with enterohepatic recycling (24-26). Finally, the MPA plasma half-life, which is > 24 hours, results in the accumulation of plasma levels of MPA and its metabolites (25,27). During steady-state conditions, this leads to only minor fluctuations during daily use, which reduces the effect of different time intervals between tablet intake and blood sampling on drug concentrations.

All steroid levels were lower than those in controls only in Group 3 patients, and their suppression was more marked in Group 3 than in Group 1. Within the dose range studied, sufficient adrenal suppression is only reached by a daily dose of 900 mg of MPA. The lack of statistical difference in steroid levels between Group 2 and 3 patients is probably caused by the small

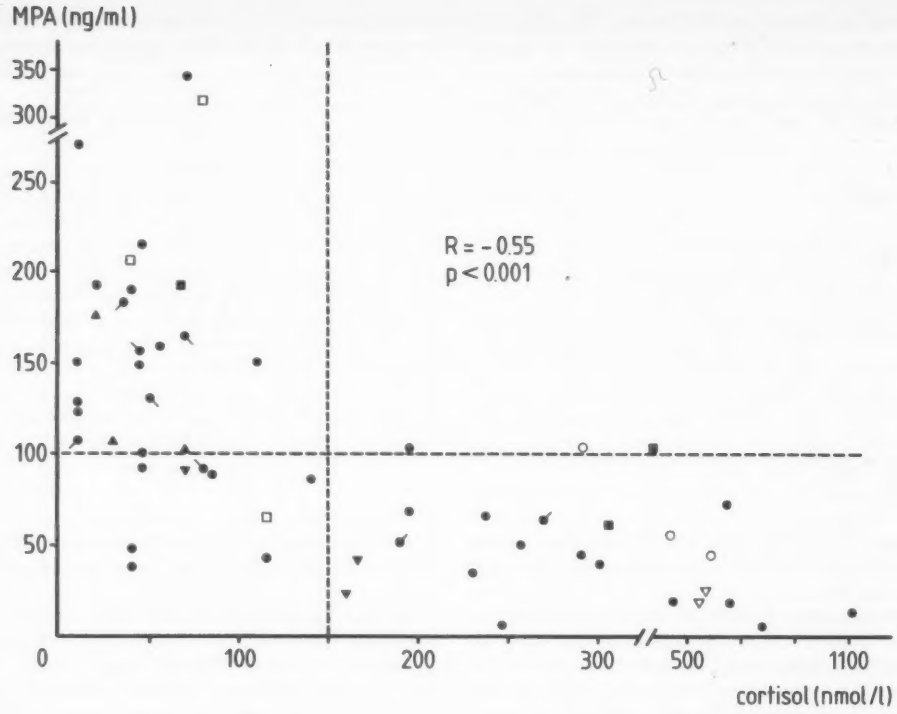

FIGURE 2.—Relation between plasma MPA concentration and plasma cortisol levels in individual samples (●). Some patients were treated with > 1 MPA dose; hormone levels in these patients are indicated by individual symbols. Horizontal dotted line signifies the minimal MPA level that has been stated to exert an effective antitumor effect, and the vertical dotted line signifies the maximal cortisol concentration after total adrenal suppression, eg, with dexamethasone.

number of patients in the former group. MPA levels are negatively correlated with those of cortisol, androstenedione, and DHAS. In patients studied on more than one dose level, the correlations are less extreme because interindividual variation is lacking. The different correlations in individual patients versus patients studied twice or three times indicate the influence of interindividual variation on these data. The main point is, however, that the negative correlation between MPA and a number of adrenal steroids is not caused by chance alone; this indicates an inhibiting influence of MPA on adrenal function.

A negative but weak correlation has been found between MPA levels and the adrenal steroids measured, but not between MPA and estrone. Some investigators have suggested a minimal MPA level, eg, 100 mg/ml in the RIA, as a criterion for adequate dosage in breast cancer treatment (5). Cortisol levels exceeding 150 nmols/L were associated with MPA concentrations < 100 mg/ml in most of our patients, which means that the latter can only reliably be used as indicator of insufficient MPA levels. Low cortisol levels, however, evidently are no proof of sufficient MPA dosage. As concerns the concerns the other steroids, DHAS appeared to correlate well with androstenedione during adrenal suppression with MPA, as we have described earlier (8). Neither of these two steroids, however, seems to have any advantage over cortisol as an indicator of adequate MPA plasma levels.

A positive correlation between androstenedione and estrone was found in the MPA-treated group. In healthy postmenopausal females, a correlation between those two parameters can also be found (11). Since androstenedione is the main source of estrone in postmenopausal women, it is not surprising that the correlation persists during suppression of this precursor steroid. The percent decrease of the median estrone level during MPA treatment is comparable with the effect obtained with other steroid blocking agents, eg, aminoglutethimide (28). The quantitative effects of both compounds on estrone levels do not seem to be essentially different. However, MPA appears to have no outspoken influence on steroid aromatization like aminoglutethimide does (29). It is clear that suppression of the main estrogen in postmenopausal life, estrone, may be of benefit in the treatment of breast cancer patients by

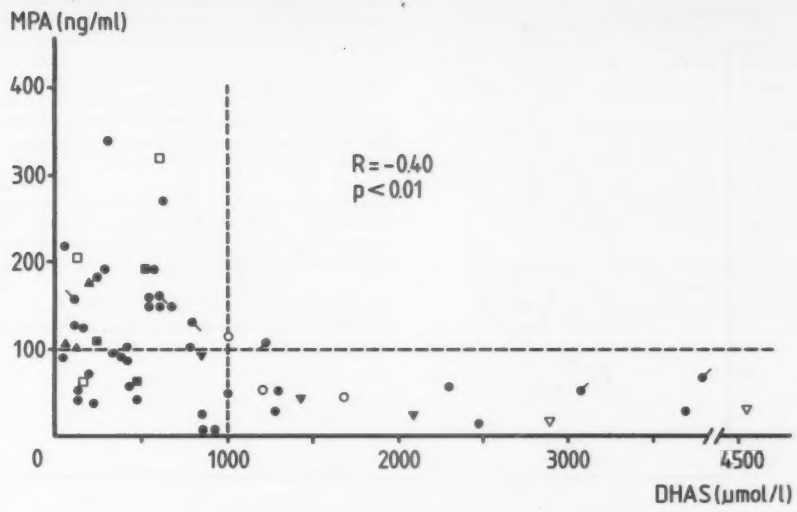

FIGURE 3.—Relation between MPA concentration and plasma DHAS levels (●). Some patients were treated with > 1 MPA dose; hormone levels in these patients are indicated by individual symbols. *See* fig 2 for further explanation.

retarding the growth of those tumor cells that are sensitive to changes in the hormonal "milieu."

The present study shows that MPA levels are higher and adrenal suppression is more pronounced with the use of 900 mg of MPA compared to a daily dosage of 300 mg. Besides, lower androstenedione and estrone levels have been found in patients using the higher dosage. This latter finding may be one of the factors which cause the greater efficacy of higher MPA dosages in the treatment of breast cancer patients, and

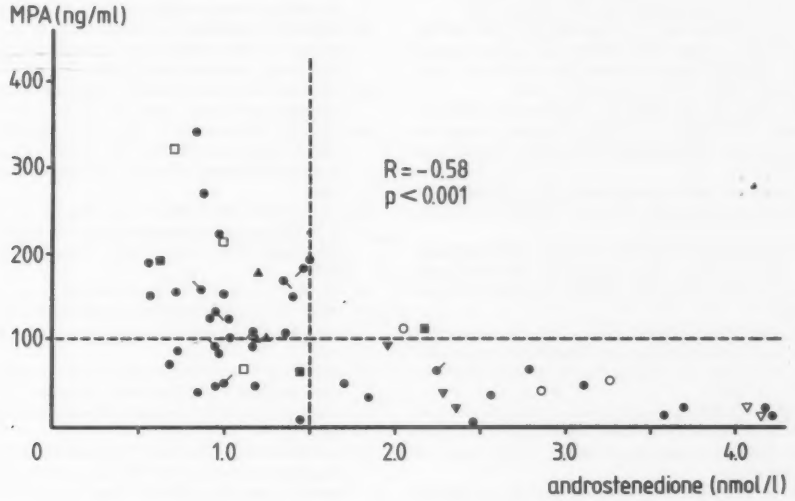

FIGURE 4.—Relation between plasma MPA concentration and plasma androstenedione levels (●). Some patients were treated with > 1 MPA dose; hormone levels in these patients are indicated by individual symbols. *See* fig 2 for further explanation.

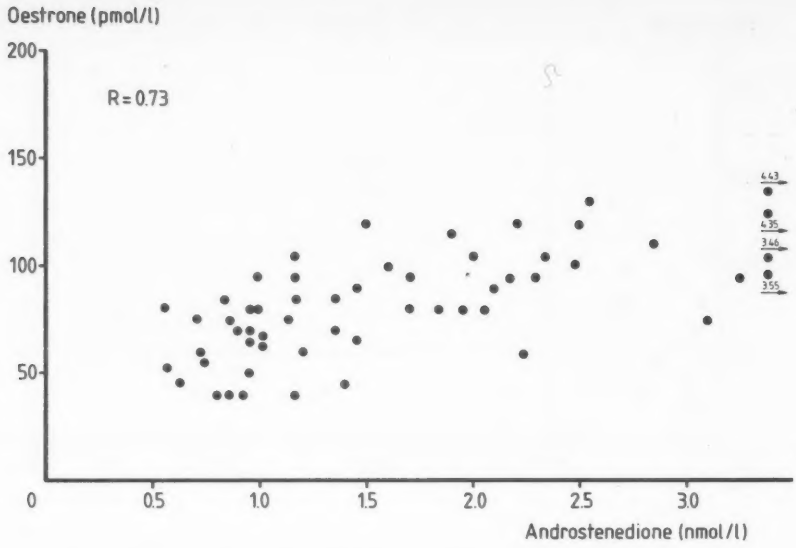

FIGURE 5.—Relation between plasma androstenedione concentration and plasma estrone levels.

thus may be one of the mechanisms by which higher MPA dosages are more effective in patients with advanced breast cancer.

REFERENCES

1. MUGGIA FM, CASSILETH PA, OCHOA M, ET AL. Treatment of breast cancer with MPA. Ann Intern Med 68:328-337, 1968.
2. GOLDENBERG IS. Clinical trial of Δ-testololactone (NSC 23759), medroxyprogesterone acetate (NSC 26386) and oxylone acetate (NSC 47438) in advanced female mammary cancer. A report of the Cooperative Breast Group. Cancer 23:109-112, 1969.
3. KLAASSEN DJ, RAPP EF, and HIRTE WE. Response to medroxyprogesterone acetate (NSC-26386) as secondary hormone therapy for metastatic breast cancer in postmenopausal women. Cancer Treat Rep 60:251-253, 1976.
4. GANZINA F. High-dose medroxyprogesterone acetate (MPA) treatment in advanced breast cancer. A review. Tumori 65:563-585, 1979.
5. TAMASSIA V, BATTAGLIA A, GANZINA F, ET AL. Pharmacokinetic approach to the selection of dose schedules for medroxyprogesterone acetate in clinical oncology. Cancer Chemother Pharmacol 8:151-156, 1982.
6. BLOSSEY HC, WANDER HE, NAGEL GA, ET AL. Medroxyprogesterone Acetat als Glucocorticoid in der Kombination mit Aminoglutethimid zur Therapie des metastasierenden Mammakarzinoms. Onkologie 5:34-41, 1982.
7. IZUO M, IINO Y, and ENDO K. Oral high-dose medroxyprogesterone acetate (MAP) in treatment of advanced breast cancer. A preliminary report of clinical and experimental studies. Breast Cancer Res Treat 1:125-130, 1981.
8. VAN VEELEN H, WILLEMSE PHB, SLEIJFER DT, ET AL. Adrenal suppression by oral high-dose medroxyprogesterone acetate in breast cancer patients. Cancer Chemother Pharmacol 12:83-86, 1984.
9. SHRIMANKAR K, SAXENA BN, and FOTHERBY K. A radioimmunoassay for serum medroxyprogesterone acetate. J Steroid Biochem 9:359-363, 1978.
10. DE LANGE WE, SLUITER WJ, PRATT JJ, ET AL. Plasma 11-deoxycortisol, androstenedione, testosterone, and ACTH in comparison with the urinary excretion of tetrahydro-11-deoxycortisol as indices of the pituitary-adrenal response to oral metapyrone. Acta Endocrinol 93:488-494, 1980.
11. POORTMAN J, THIJSSEN JHH, and DE WAARD F. Plasma oestrone, oestradiol and androstenedione levels in post-menopausal women: relation to body weight and height. Maturitas 3:65-71, 1981.
12. PANNUTI F, MARTONI A, CAMEGGI CM, ET AL. High-dose MPA in oncology. *In* Proceedings of the International Symposium on MPA. Amsterdam, Excerpta Medica, 1982, pp 5-43.
13. MATTSON W. Current status of high dose progestin treatment in advanced breast cancer. Breast Cancer Res Treat 3:231-235, 1983.
14. KAMBY C, ENGELSMAN E, and MOURIDSEN HT. Treatment of advanced breast cancer with MPA: a phase III trial. *In* Proceedings of the 2nd European Conference on Clinical Oncology. Amsterdam, 1983.
15. BLOSSEY HC, BARTSCH HH, KANNE D, ET AL. The pharmacokinetics of high-dose medroxyprogesterone acetate (MPA) in the therapy of advanced breast cancer. Cancer Chemother Pharmacol 8:77-81, 1982.
16. PANNUTI F, CAMAGGI CM, STROCCHI E, ET AL. Medroxyprogesterone acetate (MAP) relative bioavailability after single high-dose administration in cancer patients. Cancer Treat Rep 66:2043-2049, 1982.
17. MILANO G, NAMER M, BOUBLIK JL, ET AL. High dose medroxyprogesterone acetate (MPA) in cancer patients: plasmatic levels determined by an HPLC method. J Steroid Biochem 19(suppl):193S, 1983.
18. LAATIKAINEN T, NIEMINEN U, ADLERCREUTZ H. Plasma medroxyprogesterone acetate levels following intramuscular or oral administration in patients with endometrial adenocarcinoma. Acta Obstet Gynecol Scand 58:95-99, 1979.
19. BESCH PK, VORYS N, ULLERY JC, ET AL. In vivo metabolism of H^3-medroxyprogesterone acetate in pregnant and nonpregnant women and in the fetus. Am J Obstet Gynecol 95:228-237, 1966.

20. SLAUNWHITE WR, JR, and SANDBERG AA. Disposition of radioactive 17α-hydroxyprogesterone, 6α-methyl-17α-acetoxy-progesterone and 6α-methylprednisolone in human subjects. J Clin Endrocrinol Metab 21:753-764, 1961.
21. HELMREICH ML, and HUSEBY RA. Factors influencing the absorption of medroxyprogesterone acetate. Steroids 2:79-95, 1965.
22. MARTIN F, JARVENGRAA P, KOSUNEN K, ET AL. Ring A reduction of medroxyprogesterone acetate (17α-acetoxy-6α-methyl-pregnone-3,20-dione) (MPA) in biological systems. J Steroid Biochem 12:491-497, 1980.
23. GUPTA C, OSTERMAN J, SANTEN R, ET AL. In vivo metabolism of progestins. V. The effect of protocol design on the estimated metabolic clearance rate and volume of distribution of medroxyprogesterone acetate in women. J Clin Endrocrinol Metab 48:816-820, 1979.
24. ADLERCREUTZ H, MARTIN F, JARVENGRAA P, ET AL. Steroid absorption and enterohepatic recycling. Contraception 20:201-223, 1979.
25. MATHRUBUTHAM M, and FOTHERBY K. Medroxyprogesterone acetate in human serum. J Steroid Biochem 14:783-786, 1981.
26. ISHIHARA M, KIRDANI RY, OSAWA Y, ET AL. The metabolic fate of medroxyprogesterone acetate in the baboon. J Steroid Biochem 7:65-70, 1976.
27. HESSELIUS I, and JOHANSSON EDB. MPA plasma levels after oral and i.m. administration in a long term study. Acta Obstet Gynecol Scand 101:65-70, 1981.
28. SANTEN RJ, SANTNER S, DAVIS B, ET AL. Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast carcinoma. J Clin Endocrinol Metab 47:1257-1265, 1978.
29. SCHWARZEL WC, KRUGGEL WG, and BRODIE HJ. Studies on the mechanism of estrogen biosynthesis. VIII. The development of inhibitors of the enzyme system in human placenta. Endocrinology 92:866-879, 1973.

Treatment With Monoclonal Antibody to a Lewis Lung Carcinoma-Associated Antigen: Different Effects on Primary Tumor and Its Metastases[1,2]

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The growth and dissemination of Lewis lung carcinoma have been analyzed following treatment of isolated tumor cells or of tumor-bearing animals with monoclonal antibody (MoAb) 135-13C, which recognizes a cell surface tumor-associated protein of 180,000 daltons. The results of this study indicate that MoAb 135-13C binds with high affinity to Lewis lung tumor cells and induces different effects on the primary tumor (20%–25% reduction of tumor weight) and its metastasis (twofold increase of lung nodule formation). Different schedules of MoAb 135-13C administration have shown that these effects are dose- and time-dependent. In particular, the maximum increase in metastasis formation is observed when the MoAb 135-13C is administered at time of systemic tumor dissemination. In vitro preincubation of tumor cells with MoAb prior to their iv injection into normal animals results in a significant increase of pulmonary metastatic nodules. [Cancer Treat Rep 69:985–991, 1985]

It is becoming increasingly apparent by experimental models that metastatic spreading and organotropism can be regulated by distinct cell surface molecules (1–5). Thus, the availability of highly specific serologic reagents [ie, monoclonal antibodies (MoAb) to plasma membrane macromolecules] offers unique possibilities to explore their roles in modulating tumor metastatic behavior.

We have used a recently developed MoAb (135-13C) to a tumor-associated antigen of murine lung carcinomas having an apparent molecular weight of 180,000 daltons (TSP-180) (6–8). We report here that while administration of MoAb 135-13C to animals induces a delay (1.2 days) in primary tumor growth of the Lewis lung carcinoma (3LL), both spontaneous and artificial metastases are significantly increased in treated versus control animals.

MATERIALS AND METHODS

Tumor

Throughout the experiments, the 3LL line was transplanted into C57BL/6 mice weighing 20–22 g. Mice were supplied by Centro Nazionale Animali da Laboratorio CNR-ENEA, Casaccia, Rome. Each animal was injected in the hind leg muscle with 0.1 ml of a cell suspension containing 2.5×10^5 viable cells as judged by trypan blue exclusion. A standard mechanical procedure was employed for preparation of the cell suspension as previously reported (9).

MoAb Production

The generation and characterization of MoAb 135-13C has been previously described (6–8). Briefly, F344 rats were immunized with the BALB/c alveolar carcinoma line 1 and immune spleen cells were fused with P3-x63-Ag8 myeloma cells by using polyethylene glycol. The specificity of 135-13C was determined by in vitro assays (8). Cells producing MoAb 135-13C were grown as an ascitic tumor. Purified antibody was prepared by precipitation of nonimmunoglobulin proteins with caprylic acid. The supernatants were dialyzed against 0.01 M PO_4 buffer and the residual contaminants were removed by batch adsorption on DEAE-cellulose (10).

[1]Received June 14, 1984; revised Jan 28, 1985; accepted Feb 8, 1985.
[2]Supported in part by CNR "Oncologia" Finalized Project No. 840078444 and by AIRC.
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The purity of IgG preparations was assessed by precipitation with antiserum to rat IgG and analysis in SDS-PAGE. Rat MoAb 135-14 and 133-13A were from two hybridomas isolated from the same fusion as 135-13C. MoAb 135-14 has no detectable cell binding activity, while MoAb 133-13A binds to a surface protein (P100) expressed in both normal and tumor cells (8). Control immunoglobulins from nu/nu ascites were purified with the same procedure as for 135-13C. $F(ab)_2$ fragment from MoAb 135-13C was prepared by pepsin digestion followed by ammonium sulfate precipitation and gel filtration to purify the product (11).

In Vivo Studies

Groups of at least 15 animals (C57BL/6 mice weighing 20-22 g) were used for each treatment schedule. The MoAb was injected ip either as ascitic fluid or as purified protein dissolved at different doses from 0.5 to 100 μg in 0.2 ml of pyrogen-free sterile saline solution. MoAb was administered at different times before and after tumor implantation.

The effects of MoAb treatment on 3LL tumor-bearing animals were assessed by evaluating the percentage of tumor weight inhibition, by observing delay in tumor growth of the primary tumors, and by measuring the percentage of variation in metastatic lung nodules (12-14). The variation in lifespan was also evaluated as an index of antitumor activity of MoAb (13). In calculation of this value, early deaths occurring within 7 days from the end of the treatment were excluded.

Indirect Immunofluorescence Assay

Five to ten micrograms of MoAb 135-13C or its $F(ab)_2$ fragment, 5-10 μg of control monoclonal rat antibodies 135-14 and 133-13A, or 2.5 μg of MoAb antimouse $H2^b$ (C9001) to class I histocompatibility antigen was added to 10^6 tumor or C57BL/6 normal cells and/or to acetone-fixed sections of normal tissues. Cell suspensions were incubated with MoAb at 4°C for 30 minutes, washed three times with cold phosphate-buffered saline (PBS) containing 0.01 M EDTA and 0.02% NaN_3, and then incubated for an additional 30 minutes in the presence of FITC antirat gamma globulin (Cappel, Cochranville, PA) that had been extensively adsorbed with a suspension of 3LL tumor cells and C57BL/6 normal erythrocytes. At the end of the incubation, the cells were washed three times before observation in fluorescent microscope. Frozen cryostat sections were treated with 5 μg of MoAb for 30 minutes at room temperature in a moist chamber. At the end of the incubation, sections were washed three times with cold PBS and incubated with preadsorbed FITC-labeled antirat gamma globulin for an additional 30 minutes. After three washes, sections were mounted in buffered glycerol and observed in a Leitz Orthoplan microscope equipped with epi-illumination.

Lung Colonization Assay

Single-cell suspensions from 3LL tumor were obtained by mechanical dissociation as previously described (9). Cells were incubated for 1 hour at 37°C with different amounts of MoAb 135-13C (10-750 μg). After the incubation, 2×10^5 viable cells (in a volume of 0.5 ml of PBS) were injected into the tail veins of untreated mice without the addition of heavily irradiated cells or microspheres (12). Lungs were removed 18 days after inoculation and fixed in Bouin's solution; the colonies were counted with the aid of a dissecting microscope.

RESULTS

Specificity of MoAb 135-13C

The specificity of MoAb 135-13C for 3LL cells was evaluated by indirect immunofluorescence assay. Data reported in table 1 show that MoAb 135-13C and its $F(ab)_2$ fragment react specifically with primary tumors and metastases of 3LL, staining about 80% of the cells, while it does not react with normal cells and tissues. The mouse MoAb C9001 ($H2^b$) reacts with 20%-30% of

TABLE 1.—MoAb 135-13C specificity assayed by indirect immunofluorescence

	Cell suspensions*				Tissues (cryostat sections)†		
	3LL		C57BL/6				
MoAb	Primary	Metastases	Thymocytes	PBL	Lung	Liver	Kidney
135-13C‡	80	80	§	§	-	-	-
135-14	§	§	§	§	-	-	-
C9001	20	20	30	10	+	-	+
133-13A	90	90	8	35	-	-	-

*Values = % of positive cells/total cells scored.
†+ = homogeneously positive; - = negative.
‡MoAb 135-13C or its $F(ab)_2$ purified fragment.
§Values < 5%.

TABLE 2.—Effect of different schedules of MoAb treatment on primary tumors of 3LL

Experimental group	Day of treatment	Dose of MoAb (ml)*	Tumor wt inhibition (%)	Average tumor wt (g ± SE)	Tumor growth delay (days)†	Increase in life-span (%)‡
Control	—	—	—	1.01 ± 0.12	—	—
MoAb 135-13C	-1	0.2	25§	0.75 ± 0.1	1.2§	-3
	3	0.2	20§	0.81 ± 0.09	1.2§	-6
	3	0.02	—	1.02 ± 0.1	—	-3
	5	0.2	16	0.85 ± 0.06	0.6	-2
MoAb 135-14	-1	0.2	7	0.94 ± 0.09	—	-3
	3	0.2	7	0.94 ± 0.01	—	-5
	3	0.02	—	1.1 ± 0.08	—	-2
	5	0.2	10	0.91 ± 0.09	0.4	-3

*0.2 or 0.02 ml of ascitic fluids/mouse was injected ip.

†Time for tumor to reach mass of 1 g. Volume corresponding to 1 g of tumor mass was reached by control mice on 14th day after tumor implantation.

‡Median survival time of control was 22 ± 1.7 days.

§Significantly different values, $P < 0.05$ vs respective controls (Student's t test).

the tumor and normal cells [peripheral blood lymphocytes (PBL)], while rat MoAb 133-13A reacts with 90% of the tumor cells and 35% of the normal cells (PBL). By contrast, MoAb 135-14 does not react with any of the tumor or normal cells and tissues tested.

Effect of MoAb on Growth of 3LL Primary Tumors

Table 2 shows data on the effect of the treatment with MoAb (135-13C and 135-14) on 3LL primary tumors. These results show that treatment of tumor-bearing animals with MoAb 135-13C has a slight effect on the growth rate of 3LL primary tumors when it is administered the day before or 3 days after tumor implantation. In these instances, the tumor weight inhibition corresponds to 25% and 20%, respectively, while the delay in tumor growth corresponds to a value of 1.2 days. Moreover, the effect on tumor growth is reduced if tumor-bearing animals are treated on Day 5 after implant (16% tumor weight inhibition and 0.6 days of tumor growth delay) and is undetectable if treatment is undertaken at later stages (Days 7 and 9 after implant) of tumor growth (data not shown). When the dose employed for in vivo treatment is reduced tenfold (0.02 ml of ascitic fluid instead of 0.2 ml), no change in the tumor growth rate can be observed. 135-13C has poor therapeutic efficacy (as demonstrated by the values of tumor growth delay and tumor weight inhibition) when compared with antineoplastic drug treatments (12-14); however, these experiments demonstrate that MoAb 135-13C has some activity at the level of primary tumors and that this activity is dependent on (*a*) the day of treatment and (*b*) the dose employed.

Effect of MoAb 135-13C on Spontaneous Metastases

The effect of MoAb treatment on secondary tumors of 3LL is shown in figure 1. The curves refer to experiments performed with 0.2 ml of 135-13C, 133-13A, and 135-14 ascitic fluids or sterile PBS at different days from tumor implantation. Figure 1 shows that there is

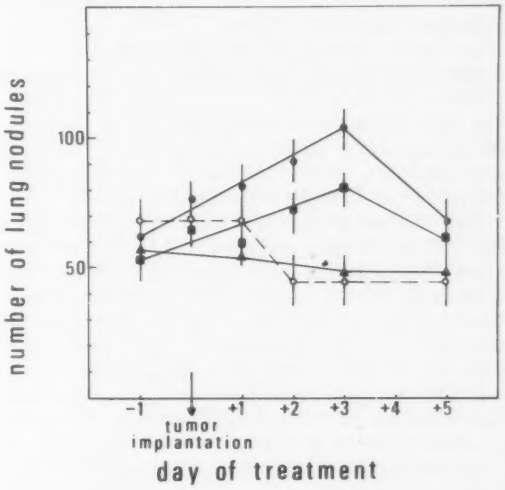

FIGURE 1.—No. of spontaneous lung metastases formations as function of day of antibody treatment of tumor-bearing mice. Viable cells (2.5×10^5) were injected im on Day 0. 0.2 ml of ascitic fluids of MoAb 135-13C (●——●), MoAb 135-14 (■——■), MoAb 133-13A (▲——▲), or 0.2 ml of PBS sterile saline solution (O----O) was injected/mouse at various days ip.

a significant increase in the number of lung nodules when MoAb 135-13C is administered to tumor-bearing animals. Particularly, it is evident that the maximum level of metastases formation is induced when MoAb 135-13C is injected on Day 3 after implant. As the figure shows, there is a progressive increase in lung nodule formation when administration of MoAb is performed within 3 days following tumor implantation, but after Day 3, there is an inversion, and at Day 5 after implant, treatment does not induce a significant change in the number of spontaneous metastases. Treatment of tumor-bearing animals with MoAb 135-14 induces some increase of metastasis formation which is statistically ($P < 0.01$) lower than that obtained by treatment with the specific MoAb (135-13C). By contrast, no effect can be observed when MoAb 133-13A is injected. On Day 3 after implant, when the maximum effect is achieved, the number of spontaneous metastases in the lung corresponds to 104 ± 8 (mean ± SE) nodules for MoAb 135-13C, 75 ±6 nodules for MoAb 135-14, 47 ± 8 nodules for MoAb 133-13A, and 45 ± 7 nodules for controls. The effect of MoAb 135-13C on spontaneous metastasis formation as a function of the dose employed is shown in figure 2. The figure represents the growth of secondary neoplasms when different doses of MoAb 135-13C are administered to tumor-bearing mice on Day 3 after implant. The values of spontaneous metastases in untreated mice 21 days after tumor implantation correspond to 45 ± 5 lung nodules. The analysis of growth curves of spontaneous metastases reported in figure 2 demonstrates an increase in the growth rate of metastases, together with a significant increase in the number of lung nodules as a function of the amount of ascitic fluid administered. The data demonstrate that the dose causing the greatest increase is 0.2 ml of ascitic fluid/mouse, inducing approximately a twofold increase of metastasis formation (108 ± 10, evaluated at Day 21 after implant).

Table 3 summarizes the effects of purified MoAb (135-13C and its $F(ab)_2$ fragment, 135-14, 133-13A, and C9001) on spontaneous lung metastases formation. The table shows that the number of lung nodules increases when low doses (0.5–1.0 μg) of MoAb 135-13C are administered, while no increase can be observed at higher doses (ie; 10, 20, or 100 μg) or when MoAb 135-14, MoAb 133-13A, and MoAb C9001 are given. The maximum enhancement of lung nodule formation (100 ± 10) is attained when 1 μg of MoAb 135-13C is injected. It is interesting to note that the overall increase in the number of spontaneous lung nodules is the same by employing optimal doses of either ascitic fluid (0.2 ml) or purified MoAb (1 μg). By contrast, it can be observed that, independently of the dose employed, no change of spontaneous lung metastasis formation occurs when the purified fragment $F(ab)_2$ of MoAb 135-13C is administered to tumor-bearing mice. These experiments clearly indicate that spontaneous lung nodule formation is strictly modulated by the timing of MoAb 135-13C administration, by the dose of MoAb administered to tumor-bearing host, and by the presence of the Fc portion of the molecule of MoAb.

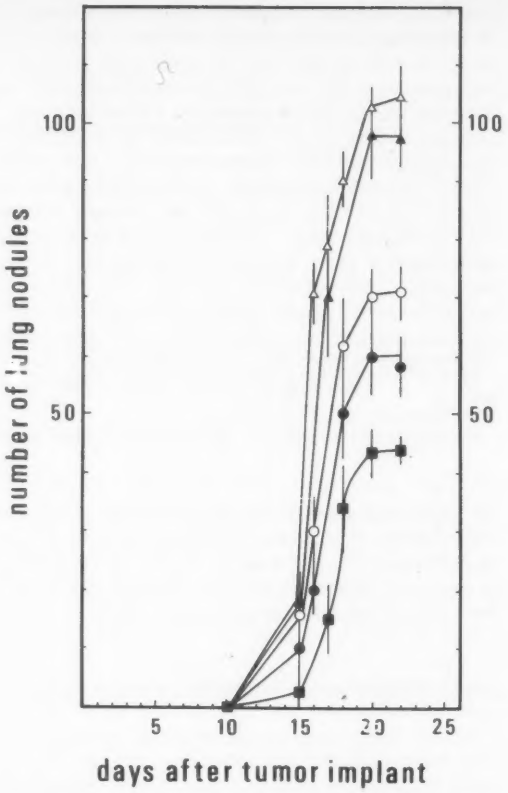

FIGURE 2.—Growth curves of spontaneous metastases in the lung of 3LL tumor-bearing mice receiving 2.5×10^5 viable cells im on Day 0. Ip treatments with various amounts of MoAb 135-13C (ascitic fluid) or saline solution were performed on Day 3 after implant. Control mice receiving 0.2 ml of PBS sterile saline solution = ■———■; mice receiving 0.02 ml of MoAb 135-13C = ●———● ; mice receiving 0.1 ml of MoAb 135-13C = ○———○; mice receiving 0.2 ml of MoAb 135-13C = △———△; and mice receiving 0.6 ml of MoAb 135-13C = ▲———▲.

Effect of MoAb 135-13C on Lung Colony Formation

Table 4 shows the effect of lung colony formation when single tumor cell suspensions are treated with MoAb 135-13C, $F(ab)_2$ of MoAB 135-13C, MoAb 135-14, MoAb 133-13A, and MoAb C9001 prior to injection into the tail veins of syngeneic mice. Data reported in the table show a direct correlation between the number of colonies arising in the lungs and the amount of puri-

TABLE 3.—Spontaneous lung metastases after im injection of 2.5×10^5 viable 3LL cells*

Treatment	Dose of MoAb (μg)	Mean No. of lung nodules ± SE	Range of lung nodules (%)	No. of mice with lung nodules/total No.
PBS†		43 ± 2	29-60 (100)	20/20
MoAb 135-13C	0.5	75 ± 5	45-163 (175)	15/15
	1.0	100 ± 10	68-122 (235)	15/15
	10.0	45 ± 7	22-66 (104)	15/15
	20.0	51 ± 4	35-77 (118)	15/15
	100.0	47 ± 9	34-68 (109)	15/15
$F(ab)_2$ fragment of MoAb 135-13C	1.0	34 ± 5	19-71 (80)	15/15
	10.0	41 ± 6	27-69 (96)	15/15
	100.0	37 ± 4	25-56 (86)	10/10
MoAb 135-14	1.0	55 ± 5	39-76 (127)	15/15
	100.0	50 ± 7	21-74 (116)	15/15
MoAb 133-13A	1.0	41 ± 5	32-70 (96)	15/15
	100.0	19 ± 7	11-61 (43)	15/15
MoAb C9001	0.5	35 ± 2	23-60 (81)	16/16
	1.0	40 ± 6	25-59 (93)	16/16
	100.0	20 ± 5	8-46 (46)	16/16

* MoAb 135-13C, $F(ab)_2$ of MoAb 135-13C, MoAb 135-14, MoAb 133-13A, MoAb C9001, or PBS was administered ip on Day 3 after implant to tumor-bearing mice.
†0.2 ml of PBS was injected/mouse.

fied antibody employed for the in vitro treatment of tumor cells. The plateau value reached after 50 μg of MoAb 135-13C treatment corresponds to approximately a twofold increase over the control value. In addition, the maximum enhancement of lung colony formation is equal for ascitic fluid and purified MoAb. Treatment of tumor cells in vitro with MoAb 135-14, 133-13A, and C9001 does not change the lung colony formation capacity of 3LL cells. Moreover, treatment of tumor cells with $F(ab)_2$ purified fragment of MoAb 135-13C induces an increase in the lung colony formation comparable to that observed when complete molecule of MoAb is employed.

DISCUSSION

Recent literature has clearly indicated that metastasis is a multistep process in which, from the cell population of the primary tumor, selected clones arise, endowed with the ability to invade host tissues and vasculature (15-17). Tumor cell surface-associated components and activities are expected to play a major role in metastatic spreading and organotropism as well as in interactions with the host immune response. Experimental evidence has been gathered so that both qualitative and quantitative changes in tumor cell surface makeup may regulate the metastatic behavior of the tumor cells (18-22).

In this context, MoAb to plasma membrane antigens have added a powerful tool in identifying tumor-associated antigens involved in the acquisition and maintenance of this property.

In the present study, we have found that MoAb 135-13C to a cell surface epitope of 3LL, which is carried on a molecule of 180,000 daltons, displays two different biological activities when administered to tumor-bearing C57BL/6 mice. A weak therapeutic effect is evident at the level of im growing primary tumors, whereas the metastatic spreading to the lungs is significantly increased. This enhancement appears to be strictly related to the metastatic process because it mainly occurs when MoAb 135-13C is given at the time when, in our experimental conditions, tumor cells would be expected to be in circulation (13,14). Furthermore, a comparable degree of enhancement can be obtained by preincubating cells in vitro with MoAb and then assaying their lung colony-forming capacity (table 4). A moderate increase, but significantly lower than that induced by MoAb 135-13C, can be observed when MoAb 135-14 (rat antibody) is administered to tumor-bearing mice (fig 1). This can be explained if we assume that rat MoAb IgG2a may react with Fc receptors on macrophages "nonspecifically," thus temporarily paralyzing the first line of defense against metastasis. In this model, 135-14 might be expected to cause a slight increase in the spontaneous metastasis formation, but, as effectively shown by results reported in table 4, is not expected to interfere with lung colony-forming capacity of 3LL tumor cells. Moreover, no enhancement of spontaneous metastases and lung colonies is found following treatment with control MoAb 133-13A and with unrelated mouse MoAb C9001.

The different effect induced by $F(ab)_2$ fragment administration in vivo to tumor-bearing mice or in vitro

TABLE 4.—Lung nodules 18 days after iv injection of 2.0×10^5 viable 3LL cells treated in vitro with MoAb 135-13C and its $F(ab)_2$ fragment, MoAb 133-13A, MoAb 135-14, or MoAb C9001

Treatment	Dose of MoAb*	Mean No. of lung nodules ± SE(%)	No. of mice with lung nodules/ total No.
PBS†		18 ± 2(100)	16/16
MoAb 135-13C	0.2 ml	38 ± 6(211)	16/16
	10 μg	25 ± 5(138)	14/14
	50 μg	34 ± 10(188)	10/10
	750 μg	36 ± 12(200)	10/10
$F(ab)_2$ fragment of MoAb 135-13C	1 μg	31 ± 9(172)	15/15
	10 μg	35 ± 5(194)	15/15
	50 μg	40 ± 6(222)	15/15
MoAb 135-14	0.5 ml	18 ± 4(100)	10/10
MoAb 133-13A	100 μg	19 ± 8(100)	15/15
MoAb C9001	100 μg	19 ± 5(100)	10/10

* Amount of MoAb given either as ascitic fluid or as purified protein.
†0.5 ml of sterile PBS was added to tumor cells incubated in vitro in complete medium.

to isolated tumor cells demonstrates that the whole molecule of MoAb 135-13C is required to induce an increase of the spontaneous lung metastases, while indicating that distinct mechanisms and steps are involved in the metastatic spreading to the lung or in the lung colony formation. The lack of lung nodule enhancement after $F(ab)_2$ injection in tumor-bearing mice can be explained, assuming that the clearance of the MoAb is very rapid when the Fc portion of the molecule is lost and/or that MoAb 135-13C by its Fc portion tightly binds tumor cells (targets) to some effector cells that are able to mediate the metastatic process. By contrast, the comparable enhancement induced by $F(ab)_2$ or by the whole molecule of MoAb on lung colony formation of isolated tumor cells demonstrates that the $F(ab)_2$ portion of MoAb 135-13C binding 3LL tumor cells alters their cell surface, thus changing their lung colony-forming capacity.

The postulate that MoAb tumor cells interact with some effector cells can also explain the lack of lung nodule enhancement when high doses of purified MoAb 135-13C (table 3) are employed. In fact, we may suppose that a large excess of MoAb molecules saturates the Fc receptors on the effector cells, blocking their binding to the tumor cells. Otherwise, it is also possible to postulate that lung nodule enhancement is mediated by bivalent binding of antibody to cell surface determinants, which leads to either cell aggregation or aggregation of TSP-180 on cell surface. Large excess results in a minimum of bivalent binding analogous to a "prozone" effect in immunoprecipitation.

The present findings clearly indicate that binding of some specific MoAb to a tumor cell surface can result in a dose-dependent increase of its metastatic capacity, but they provide little insight into the mechanisms responsible for this enhancement. The possibility that the phenomenon is mediated by a mere perturbation of the cell surface architecture or by tumor cell agglutination seems unlikely, in view of the finding that injection in the tumor-bearing animals or incubation of the tumor cells with MoAb C9001 to class I $H2^b$ histocompatibility antigens or with MoAb 133-13A (recognizing a P100 surface protein present on both normal and tumor cells) is unable to induce metastatic enhancement (fig 1; tables 3 and 4), and no agglutination was detectable microscopically. Although immune mechanisms cannot be completely excluded (ie, perturbation of NK cell network), they are likely to have a minor role in this experimental model, as suggested by the time relationship of the MoAb injection and the stage of tumor growth. Although not appreciated immunohistochemically, radiolabeled MoAb 135-13C appears to concentrate in the lung vasculature (23); thus, one can hypothesize that the antibody can somehow change vessel permeability, thus favoring metastasization. Yuhas et al (24) observed that inhibition of primary tumor growth can stimulate growth of metastasis of line 1 tumors. Although similar observations have not been reported for 3LL tumors, such a mechanism might be operable here. Despite the fact that the present study can have limited general biological significance, these findings indicate that the cell surface molecules exist on tumor cells which can be modulated with resulting changes in cell metastatic properties. This is of particular interest in view of the prospective use of MoAb in the diagnosis and control of human neoplasms.

REFERENCES

1. RIEBER M, and RIEBER MS. Metastatic potential correlates with

cell surface protein alterations in B16 melanoma variants. Nature 293:76-79, 1981.
2. LE GRUE SJ, and HEARN G. Extraction of immunogenic and suppressogenic antigens from variants of B16 melanoma exhibiting low and high metastatic potential. Cancer Res 43:5106-5111, 1983.
3. MINER KN, KAWAGUCHI T, UBA GW, ET AL. Clonal drift of cell surface, melanogenic and experimental metastatic properties of in vivo selected brain meninges-colonizing murine B16 melanoma. Cancer Res 42:4631-4638, 1982.
4. NICOLSON GL. Cell surface and cancer metastasis. Hosp Pract 10:75-83, 1982.
5. FOGEL M, GORELIK E, SEGAL S, ET AL. Differences in cell surface antigens of tumor metastases and those of the local tumor. JNCI 62:585-588, 1979.
6. KENNEL SJ. Characterization of tumor cell surface protein with heterologous antisera to a spontaneous Balb/c lung carcinoma. Cancer Res 39:2934-2939, 1979.
7. KENNEL SJ, LANKFORD PK, FOOTE LJ, ET AL. Distribution of a tumor cell surface protein common to several murine lung carcinomas. Cancer Res 40:2153-2159, 1980.
8. KENNEL SJ, FOOTE LJ, and LANKFORD PK. Analysis of surface proteins of mouse lung carcinomas using monoclonal antibodies. Cancer Res 41:3465-3470, 1981.
9. STARACE G, BADARACCO G, GRECO C, ET AL. DNA distribution of in vivo and in vitro lines of Lewis lung carcinoma. Eur J Cancer 18:973-978, 1982.
10. STEINBUCH M, and ANDRAN R. The isolation of IgG from mammalian sera with the aid of caprylic acid. Arch Biochem Biophys 131:279-284, 1969.
11. KENNEL SJ. Binding of monoclonal antibody to protein antigen in fluid phase or bound to solid supports. J Immunol Methods 55:1-12, 1982.
12. SACCHI A, CALABRESI F, GRECO C, ET AL. Different metastatic potential of in vitro and in vivo lines selected from Lewis lung carcinoma: correlation with response to different bleomycin schedulings. Invasion Metastasis 1:227-238, 1981.
13. GRECO C, CALABRESI F, CAPUTO M, ET AL. Adriamycin effect on the Lewis lung carcinoma comparison between the original and its derivative subline. Eur J Cancer 16:1251-1255, 1980.
14. ZUPI G, CAVALLARI A, GRECO C, ET AL. Lewis lung carcinoma lines with different metastatic behavior: response to ICRF-159. Anticancer Res 4:91-95, 1984.
15. NICOLSON GL. Cancer metastasis: organ colonization and cell surface properties of malignant cells. Biochem Biophys Acta 695:113-176, 1982.
16. FIDLER IG, and KRIPKE ML. Metastasis results from preexisting variant cells within a malignant tumor. Science 197:893-895, 1977.
17. TALMADGE JE. The selective nature of metastasis. Cancer Metastasis Rev 2:25-40, 1983.
18. SHEARMAN PL, and LONGENECKER BM. Clonal variation and functional correlation of organ. Specific metastasis and organ-specific metastasis associated antigen. Int J Cancer 27:387-395, 1981.
19. ALTEVOGT P, FOGEL M, CHEINGSANG-POPOV R, ET AL. Different patterns of lectin binding and cell surface syalynation detected on related high-and-low-metastatic tumor lines. Cancer Res 43:5138-5144, 1983.
20. KATZAV S, DE BAETSELIER P, TARTAKOSKY B, ET AL. Alterations in major histocompatibility complex phenotypes of mouse cloned T10 sarcoma cells: association with shifts from nonmetastatic to metastatic cells. JNCI 71:317-324, 1983.
21. ISAKOV N, KATZAV S, FELDMAN M, ET AL. Loss of expression of transplantation antigens encoded by the H2k locus on Lewis lung carcinoma cells and its relevance to the tumor's metastatic properties. JNCI 71:139-145, 1983.
22. EISENBACH L, SEGAL S, and FELDMAN M. MHC imbalance and metastatic spread in Lewis lung carcinoma clones. Int J Cancer 32:113-120, 1983.
23. KENNEL SJ, FLYNN K, FOOTE LJ, ET AL. Monoclonal antibodies in cancer detection and therapy. Bioscience 34:150-156, 1984.
24. YUHAS JM, TOYA RE, and WAGNER E. Specific and non specific stimulation of resistance to the growth and metastasis of the line 1 lung carcinoma. Cancer Res 35:242-244, 1975.

Factors Modifying Cytotoxicity Induced by 5-FU and Hydroxyurea[1,2]

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Pretreatment of exponentially growing 9L cells for 24 hours with a nontoxic (0.77 μM) or moderately toxic (7.7 μM) concentration of 5-FU (5-FUra) enhanced cell kill induced by a subsequent 6-hour treatment with 1.3 mM hydroxyurea (HU) by a magnitude of 1 log. In confluent 9L cultures or 9L cells treated in serum-free medium that decreased the cycling population, however, the 5-FUra-HU combination did not enhance the cell kill. No recovery from potentially lethal damage was observed for up to 24 hours after treatment of 9L cells with 7.7 μM 5-FUra alone or with 0.77 or 7.7 μM 5-FUra followed by 1.3 mM HU. These results show that 5-FUra and HU in combination kill cycling cells effectively and that cytotoxicity is fixed on completion of the treatment. Although the number of sister chromatid exchanges per metaphase increased significantly with increasing length of exposure to a nontoxic (0.77 μM) or moderately toxic (7.7 μM) concentration of 5-FUra, subsequent treatment with HU that resulted in cell kills of 70%–80% and 99%, respectively, did not increase the number of sister chromatid exchanges per metaphase beyond that expected from 5-FUra treatment alone. Thus, the enhanced cell kill does not appear to be a result of direct DNA damage. [Cancer Treat Rep 69:993–997, 1985]

Previous studies from our laboratory have shown that treatment of exponentially growing 9L rat brain tumor cells with a nontoxic concentration (0.77 μM) of 5-FU (5-FUra) resulted in the accumulation of S-phase cells, whereas a moderately toxic but relatively low concentration of 5-FUra (7.7 μM) produced a partial G1-S block that caused cells to accumulate in the G1 phase (1,2). When 9L cells were treated with the S-phase-specific agent hydroxyurea (HU) (1.3 mM) immediately after treatment with 5-FUra at either concentration, an enhanced cell kill resulted (2). Cells treated with different concentrations of 5-FUra demonstrated different cytokinetic perturbation patterns; therefore, the enhanced cell kill did not result simply from synchronization of cells in G1 or S phase or from the phase specificity of HU.

A similar cell kill was observed when a 24-hour treatment with a nontoxic or moderately toxic dose of 5-fluorodeoxyuridine (5-FdUrd), 5,8-dideazaisofolic acid, or 2′-deoxy-2′-fluoro-ara-uracil was followed by treatment with 1.3 mM HU for 6 hours (3). When a similar dose of HU or 1-formylisoquinoline thiosemicarbazone was administered for 6 hours immediately after a 24-hour treatment with either 0.77 or 7.7 μM FUra, a cell kill of approximately 1 log in addition to that caused by each drug alone was obtained (3). Thus, the cell kill was consistently increased when a low dose of thymidylate synthetase inhibitor was administered 24 hours before a 6-hour treatment with a low dose of an agent that acts as a ribonucleotide reductase inhibitor.

Because low serum levels of 5-FUra can be achieved in humans (4) and a serum HU level of 0.26–5.0 mM has been obtained with a fractionated daily dose of oral HU [180–400 mg/kg (5)], use of 5-FUra in combination with HU to treat patients with malignant neoplasms seems logical. Whether such clinical protocols will be successful depends on various factors. One such factor is the relative response of cycling and noncycling tumor cells to the combined treatment. In addition, the population of cycling cells includes cells in all phases of the cell cycle, and therefore the differential sensitivity exhibited by these cells, and the fraction of cells in each phase, will also affect the response to therapy. Another factor is the ability of tumor and normal cells to recover from damage produced by either or both drugs. In this report, we describe

[1]Received July 27, 1984; revised Mar 4, 1985; accepted Mar 15, 1985.

[2]Supported in part by grant PDT-159 from the American Cancer Society, and by Public Health Service grant CA-19992 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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[4]We thank Dennis F. Deen, PhD, for his excellent comments and suggestions, Tania Retivov for manuscript preparation, and Stephen Ordway for editorial assistance.

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experiments in which several factors that modify the cell kill induced by combination treatment with 5-FUra and HU were investigated in 9L cells using colony-forming efficiency (CFE) and sister chromatid exchange (SCE) assays to determine whether the enhanced cell kill was the result of increased DNA damage.

MATERIALS AND METHODS

9L Cell Culture

Monolayer cultures of 9L cells were grown in complete medium consisting of Eagle's minimum essential medium (CMEM) supplemented with nonessential amino acids, 10% newborn calf serum (NBCS), and gentamicin (50 μg/ml) in a humidified mixture of 95% air and 5% CO_2 at 37°C.

Exponentially growing cultures were obtained by seeding 1×10^6 cells into 75-cm^2 tissue culture flasks (Corning, Oxnard, CA) containing CMEM 48 hours before the drug treatment. At the time of treatment, each flask contained approximately 3.5×10^6 cells. Nutrient-deficient cultures were obtained by replacing the CMEM with Earle's balanced salt solution (EBSS) 2 hours before treatment. Confluent cultures were obtained by seeding 1×10^6 exponentially growing cells into 75-cm^2 tissue culture flasks containing CMEM, where they grew unfed until the experiments were performed 5 days later. Under these conditions, the number of cells per flask increased exponentially during the first 3 days and then reached a plateau of approximately 1.6×10^7 cells/flask on Day 4, where it remained for at least 3 more days.

Cell Survival Assay

Cell survival was determined with a CFE assay (6,7). Surviving fractions (SF) were calculated as the ratio of the CFEs of treated cells to the CFEs of control cells. When the two drugs were combined, the expected additive cell kill was estimated as the product of the SFs of each drug acting alone (8).

Drugs and Treatments

Stock solutions of 5-FUra (Roche Laboratories, Nutley, NJ) and HU (Calbiochem-Behring, La Jolla, CA) were added to cells at different volumes to achieve the desired final concentrations. The 9L cells were treated with 5-FUra at one of two concentrations: 0.77 μM, which is noncytotoxic, and 7.7 μM, which produces 85% cell kill; 1.3 mM HU kills approximately 20%-30% of the cells (2). For combination treatments, CMEM or EBSS that contained the first drug was decanted, and the cells were rinsed twice with prewarmed MEM and then refed with CMEM or EBSS containing the second drug.

Recovery From Potentially Lethal Damage

After the drug treatments, one group of control cells and one group of treated cells were plated immediately to determine colony formation. Other groups of control and treated cells were washed twice with EBSS and allowed to recover for up to 24 hours in EBSS in the humidified incubator at 37°C. After various recovery times, cells were trypsinized and plated to determine cell survival. All experiments were repeated at least three times.

SCE Assay

After 12-30 hours of treatment with 0.77 or 7.7 μM 5-FUra or after a 24-hour exposure to 0.77 μM 5-FUra followed immediately by a 6-hour exposure to 1.3 mM HU, the cells were rinsed and 25 ml of CMEM containing 5 μM bromodeoxyuridine (Sigma Co, St. Louis, MO) was added. The cells were allowed to replicate for two cell cycles (approximately 30-40 hours) and were then treated with demecolcine at a dose of 0.4 μg/ml (Sigma Co) for 4 hours. Mitotic cells were shaken from the flasks, treated with 0.05 M KCl for 10 minutes, and fixed with freshly prepared Carnoy's solution. Sister chromatids were differentially stained using the method of Perry and Wolff (9), and SCEs were counted in 20 metaphase cells in each group.

Flow Cytometric Analysis

Approximately 1×10^6 cells in suspension were fixed with 70% ethanol, stained with chromomycin A_3 (Calbiochem-Behring), and suspended in distilled water for flow cytometric analysis (10) with a FACS III flow cytometer (Becton-Dickinson, Sunnyvale, CA). DNA histograms were obtained from 5×10^4 cells and analyzed using Dean's computer program (11).

RESULTS

Effect of EBSS on the 5-FUra-HU Combination

Immediately after CMEM was replaced with EBSS, the cells stopped growing and there was no obvious cell death for the next 24 hours; CFEs of untreated controls in either CMEM or EBSS were approximately 65% throughout this period. Cytotoxicity against cells treated in EBSS was markedly reduced in cells treated with 5-FUra alone and in those treated with the 5-FUra-HU combination (fig 1). Although cytokinetic perturbations similar to those reported previously (1,2) were observed in cells treated in CMEM, the cells treated with 0.77 or 7.7 μM 5-FUra in EBSS showed a gradual reduction in the number of S-phase cells over the 24-hour period (from 45% to

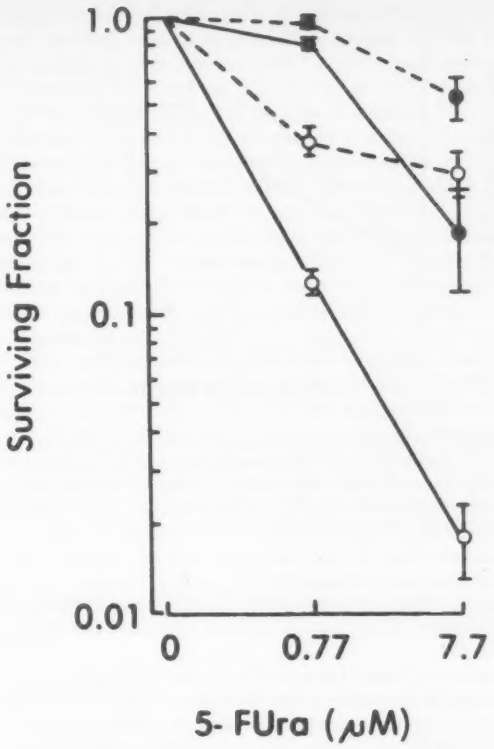

FIGURE 1.—Dose-response curves for 9L cells treated with 5-FUra alone for 24 hrs (filled circles) or with 5-FUra for 24 hrs and HU for 6 hrs (open circles) in CMEM (solid lines) or in EBSS (broken lines).

35%). This change was also observed in untreated cells kept in EBSS.

Drug Combination on Confluent Cultures

The SFs of cells in confluent conditions treated with 5-FUra or 5-FUra-HU increased considerably compared with cells in exponential growth (fig 2). DNA histograms of confluent cultures treated with either 0.77 or 7.7 μM 5-FUra were the same as the histogram of the untreated confluent culture: all showed very low numbers of cells in S and G2/M phases throughout the 24-hour time course.

Recovery From Potentially Lethal Damage

No recovery from potentially lethal damage (PLD) was observed for up to 24 hours after treatment in 9L cells treated with 7.7 μM 5-FUra alone or in those treated first with 0.77 or 7.7 μM 5-FUra and then with 1.3 mM HU (fig 3).

SCE Assay

Control cultures had an average of 12 ± 3.4 (SD) SCEs/metaphase. When 9L cells were treated with 0.77 μM 5-FUra (nontoxic concentration up to 24 hours) or 7.7 μM 5-FUra (toxic concentration, with up to 80% cell kill at 24 hours), the number of SCEs per metaphase was greater at all time points than in control cultures ($P < 0.001$); at both concentrations, however, the number of SCEs reached a plateau after 18 hours of treatment. In cells treated for 18 and 24 hours with 0.77 or 7.7 μM 5-FUra, additional treatment with 1.3 mM HU for 6 hours resulted in an increased cell kill of 1 log, but did not increase the number of SCEs per metaphase beyond that expected from 5-FUra treatment alone (fig 4). Thus, the number of SCEs induced by 5-FUra is time-dependent, but independent of cell kill induced by 5-FUra treatment alone or in combination with HU.

DISCUSSION

Preincubation of exponentially growing 9L cells with a

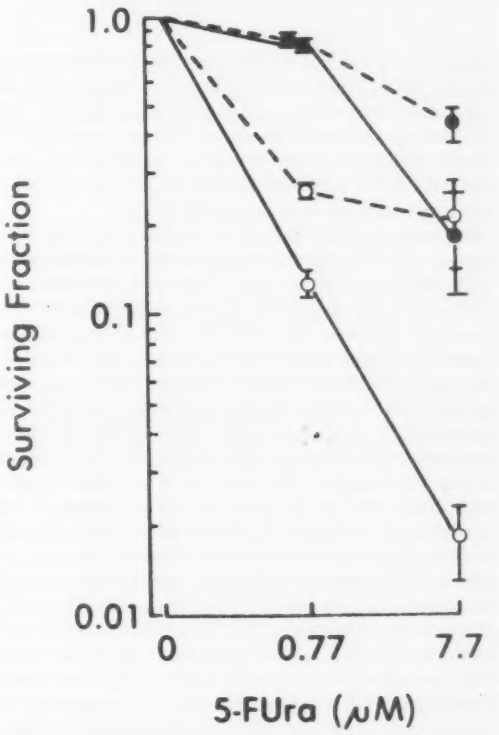

FIGURE 2.—Dose-response curves for 9L cells treated with 5-FUra alone for 24 hrs (filled circles) or with 5-FUra for 24 hrs and HU for 6 hrs (open circles). Solid lines indicate cells cultured in CMEM; broken lines indicate cells in confluent cultures.

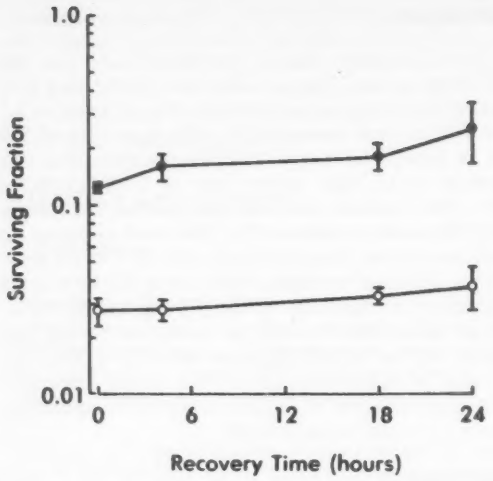

FIGURE 3.—Recovery expressed as surviving fraction for exponentially growing 9L cells treated for 24 hrs with 0.77 μM (filled circles) or 7.7 μM (open circles) 5-FUra followed by treatment with 1.3 mM HU for 6 hrs and held for various times before trypsinization in EBSS at 37°C.

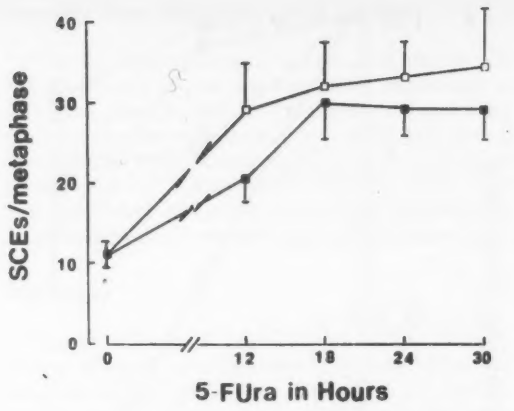

FIGURE 4.—SCEs/metaphase in cells treated with 5-FUra (filled squares, 0.77 μM; open squares, 7.7 μM) for various durations. HU treatment after pretreatment with 5-FUra (0.77 or 7.7 μM) for 24 hrs did not increase the number of SCEs (31.2 and 34.5, respectively) beyond that expected from treatment with 5-FUra alone.

nontoxic or a low but moderately toxic concentration of 5-FUra for 24 hours has been found to enhance the cell kill induced by a subsequent 6-hour treatment with HU (2). Because the serum levels of 5-FUra (4) and HU (5) used in the present study can be achieved clinically, several factors that may be important to the effectiveness of this drug combination were studied further.

One such factor is whether this combination drug treatment is effective only against cycling cells or whether it is also effective against noncycling cells. Confluent cell cultures provide one model of noncycling cells in vivo. However, cells also usually stop cycling when normal growth medium is replaced with nutrient-deficient medium, such as EBSS or depleted medium. Noncycling cells derived in this fashion resume proliferation when the cell population is reduced or when fresh CMEM is added. Confluent cell cultures usually consist of a large diploid population, whereas cells growing in medium replaced with EBSS cease proliferation at various phases of the cell cycle (12). Although we do not know which kinds of in vitro noncycling cells resemble those in vivo, our results do show that the 5-FUra-HU combination is not effective against both kinds of noncycling cells. Therefore, cells apparently blocked at the G1-S border by prolonged administration of 7.7 μM 5-FUra may not be similar to G0 cells, but instead have already initiated DNA synthesis; however, the DNA synthesis pathway is suppressed at such an early state that we cannot detect these S-phase cells by flow cytometry or autoradiography. Thus, the mechanism of the enhanced cell kill with the 5-FUra-HU combination might be interpreted as some biological events related to suppression of activated thymidylate synthetase followed by blocking of ribonucleotide reductase (3).

Recovery from PLD is another factor that might be important in determining the effectiveness of drugs used in combination therapy (13). The concept of PLD and recovery from PLD was first proposed for radiation damage, but as far as we know, the actual molecular events leading to these phenomena remain unclear (13). Nonetheless, recovery from PLD in experimental systems can often affect cell kill by an order of magnitude or more. If recovery from PLD occurs in human tumors, it certainly could be a major determinant of the success or failure of a particular therapy. Our in vitro studies were performed to help determine whether recovery from PLD induced by 5-FUra and HU might also prove useful in evaluating chemotherapeutic regimens, as the CFE assay requires trypsinization, which does not occur in vivo.

In the present study, 9L cells incubated in EBSS after treatment with 5-FUra-HU did not recover from PLD for up to 24 hours after treatment. Whereas the recovery from PLD induced by spirohydantoin nitrogen mustard in 9L cells may be responsible for the relative resistance of 9L multicellular spheroids or 9L tumors in situ to treatment with that agent (7,14), the effectiveness of carmustine (BCNU) in treating 9L cells in multicellular spheroids (15) or in situ (16,17) may be related to the lack of recovery from BCNU-induced PLD in this cell line, similar to the lack of recovery from PLD after treatment with 5-FUra-HU.

The SCE assay is a rapid and sensitive method for measuring the effects of agents that damage DNA (18). Al-

though the actual mechanism of SCE induction has not been clarified, cross-linking of DNA strands appears to be one of the events involved (19). Yet, other antineoplastic drugs such as busulfan (20), vincristine (20), cisplatin (21), and cyclophosphamide (22), some of which are not cross-linking agents, are also reported to induce SCEs. It is interesting that the nontoxic concentration of 5-FUra induced SCEs, but addition of HU that actually resulted in additional cell kill did not cause an additional increase in SCEs. It is not known whether 5-FUra induces such cross-linking, but it does damage DNA in a way that increases the number of SCEs at both toxic and nontoxic doses; this increase is time-dependent, but is independent of the magnitude of cell kill.

Thus, our results demonstrate that treatment with 5-FUra and HU in combination can kill cycling cells effectively and that cytotoxicity is fixed on completion of treatment. In addition, the enhanced cell kill obtained with HU after treatment with 5-FUra may result from some biochemical interaction not directly related to DNA damage that is manifested by SCEs, such as cross-linking. The usefulness of this treatment should be enhanced when coordinated with administration of cell cycle-nonspecific agents such as the chloroethyl nitrosoureas, which kill noncycling neoplastic cells, at appropriate intervals.

REFERENCES

1. WEIZSAECKER M, HOSHINO T, DEEN DF, ET AL. Cytokinetic and cytotoxic effects of antimetabolites on 9L rat brain tumor cells in vitro. Cancer Res 41:3835-3839, 1981.
2. KOBAYASHI S, and HOSHINO T. Combined cytotoxic effect of low-dose 5-fluorouracil and hydroxyurea of 9L cells in vitro. Cancer Res 43:5309-5313, 1983.
3. KOBAYASHI S, HOSHINO T, and SANTI DV. Experimental combination chemotherapy with thymidylate synthetase and ribonucleotide reductase inhibitors. Pharm Res 1:181-183, 1984.
4. HORNBECK CL, FLOYD RA, BYFIELD JE, ET AL. Cerebrospinal fluid versus serum concentrations of 5-FU, allopurinol, and oxypurinol during treatment of metastatic brain cancers with 5-FU infusion, allopurinol, and radiation. Cancer Treat Rep 66:571-573, 1982.
5. CREASEY WA, CAPIZZI RL, and DECONTI RC. Clinical and biochemical studies of high-dose intermittent therapy of solid tumors with hydroxyurea (NSC-32065). Cancer Chemother Rep 54:191-194, 1970.
6. DEEN DF, BARTLE PM, and WILLIAMS ME. Response of cultured 9L cells to spirohydantoin mustard and x-rays. Int J Radiat Oncol Biol Phys 5:1663-1667, 1979.
7. DEEN DF, HOSHINO T, WILLIAMS ME, ET AL. Response of 9L tumor cells in vitro to spirohydantoin mustard. Cancer Res 39:4336-4340, 1979.
8. VALERIOTE F, and LIN H. Synergistic interaction of anticancer agents: a cellular perspective. Cancer Chemother Rep 59:895-900, 1973.
9. PERRY P, and WOLFF S. New Giemsa method for differential staining of sister chromatids. Nature 251:156-158, 1974.
10. CRISSMAN HA, and TOBEY RA. Cell cycle analysis in 20 minutes. Science 184:1297-1298, 1974.
11. DEAN PN. Simplified methods of analyzing DNA-distributions from perturbed cell populations. Cell Tissue Kinet 13:672, 1980.
12. BERTRAND M, DEEN DF, HOSHINO T, ET AL. Recovery from potentially lethal damage induced by spirohydantoin mustard on 9L cells in vitro. Cancer Treat Rep 64:889-895, 1980.
13. BERTRAND M, and DEEN DF. Factors influencing the recovery from potentially lethal damage (PLD) in mammalian cells in vitro and in vivo. Cancer Treat Rev 7:1-15, 1980.
14. SANO Y, HOSHINO T, BJERKVIG R, ET AL. The relative resistance of non-cycling cells in 9L multicellular spheroids to spirohydantoin mustard. Eur J Cancer Clin Oncol 19:1451-1456, 1983.
15. DEEN DF, HOSHINO T, WILLIAMS ME, ET AL. Development of a 9L rat brain tumor cell multicellular spheroid system and its response to 1,3-bis(2-chloroethyl)-1-nitrosourea and radiation. JNCI 64:1373-1382, 1980.
16. ROSENBLUM ML, GEROSA MA, DOUGHERTY DV, ET AL. Improved treatment of a brain-tumor model. Part 1: advantages of single- over multiple-dose BCNU schedule. J Neurosurg 58:177-182, 1983.
17. GEROSA MA, DOUGHERTY DV, WILSON CB, ET AL. Improved treatment of a brain-tumor model. Part 2: sequential therapy with BCNU and 5-fluorouracil. J Neurosurg 58:368-373, 1983.
18. WOLFF S. Measurement of sister chromatid exchanges in mammalian cells. *In* DNA Repair: A Laboratory Manual of Research Procedures, Part B (Friedberg EC, and Hanswalt PC, eds). New York, Marcel Dekker, 1981, vol 1, pp 577-585.
19. TOFILON PJ, WILLIAMS ME, and DEEN DF. Nitrosourea-induced sister chromatid exchanges and correlation to cell survival in 9L rat brain tumor cells. Cancer Res 43:473-475, 1983.
20. RAPOSA T. Sister chromatid exchange studies for monitoring DNA damage and repair capacity after cytostatics in vitro and in lymphocytes of leukaemic patients under cytostatic therapy. Mutat Res 57:241-251, 1978.
21. BRADLEY MO, HSU IC, and HARRIS CC. Relationships between sister chromatid exchanges and mutagenicity, toxicity, and DNA damage. Nature 282:318-320, 1979.
22. YANG DP, GRAUPENSPERGER F, MINECCI LC, ET AL. Induction of sister chromatid exchanges in human diploid fibroblasts by mutagens with and without rat liver microsomal activation. Environ Mutagen 3:45-52, 1981.

Combination of Dacarbazine and Doxorubicin in the Treatment of Childhood Rhabdomyosarcoma[1,2]

Erlinda Etcubanas,* Marc Horowitz, and Ralph Vogel[3,4]

The combination of dacarbazine and doxorubicin was given to 26 children with untreated rhabdomyosarcoma to determine its efficacy as frontline chemotherapy. A treatment course consisted of 250 mg/m^2 of dacarbazine given iv on Days 1–5 and 60 mg/m^2 of doxorubicin given iv on Day 1. After three courses of therapy, 17 patients (65%) achieved partial response and nine failed to respond. The side effects of treatment consisted of nausea, vomiting, flu-like symptoms, neutropenia associated with fever, mucositis, and thrombocytopenia (rarely). Although the response rate is comparable to other drug combinations, the lack of complete responses to the combination indicates that it is less effective as frontline therapy. [Cancer Treat Rep 69:999–1000, 1985]

The conventional agents used for the treatment of childhood rhabdomyosarcoma are vincristine, cyclophosphamide, doxorubicin, and dactinomycin (1). To increase the number of effective drugs for the treatment of childhood rhabdomyosarcoma and to possibly improve response rates, we investigated the efficacy of doxorubicin combined with darcarbazine (DTIC) as induction therapy. This choice was based on reports of the efficacy of the two drugs in adult soft tissue sarcoma, the combination being superior to either drug alone (2). Cangir et al (3) reported five of 13 responses (one complete and four partial) to this drug combination in children with refractory rhabdomyosarcoma. This drug combination has not been adequately investigated for use as frontline therapy in childhood rhabdomyosarcomas; therefore, we included the combination as the first component of treatment within the framework of a multimodal treatment protocol for childhood rhabdomyosarcoma. This report deals solely with responses to DTIC and doxorubicin as induction chemotherapy.

PATIENTS AND METHODS

Twenty-six children were entered in the study. Eligibility requirements included histologically proven rhabdomyosarcoma and the presence of measurable disease. Fourteen patients had localized or regional disease and 12 had disseminated disease.

Treatment Schedule

Doxorubicin (60 mg/m^2) was given iv on Day 1; DTIC (250 mg/m^2) was given iv on Days 1–5, and was repeated every 21 days. Responders continued to receive this drug combination during maintenance therapy in alternating sequence with combinations of vincristine, cyclophosphamide, and dactinomycin.

Evaluation of Response

Responses were assessed after three treatment courses (Day 60) unless there was tumor progression, in which case treatment was discontinued and the patient was classified as a nonresponder. Complete response was defined as the complete disappearance of all measurable lesions; partial response was defined as a $\geq$ 50% decrease in the tumor volume; and no response was defined as a $<$ 50% decrease in tumor volume.

RESULTS

All patients were eligible for evaluation of response

[1]Received Mar 26, 1984; revised Feb 6, 1985; accepted Feb 19, 1985.

[2]Supported by Public Health Service grants CA-21765 and CA-23099 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by the American Lebanese Syrian Associated Charities.

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[4]We thank J. R. Gilbert for editorial suggestions in the preparation of the manuscript, and M. Cain and L. Avery for their assistance with clinical data management.

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and toxicity. Treatment was discontinued in two patients because of progressive disease. Thus, 74 of the 78 planned treatment courses were delivered.

Response to Treatment

Seventeen of 26 (65%) patients had partial responses, ten of 14 with localized or regional disease and seven of 12 with metastatic disease. Because responders subsequently received other drug combinations, the duration of response to doxorubicin and DTIC could not be determined.

Toxicity

Nausea and vomiting occurred in all patients, usually on the first day of treatment. Of 74 treatment courses given, ten resulted in fever and neutropenia, five resulted in mucositis, four resulted in flu-like symptoms (4), and one resulted in thrombocytopenia.

DISCUSSION

Our results (17 patients with partial responses and nine with no response, for a favorable response rate of 65%) demonstrate the activity of the combination of DTIC and doxorubicin in childhood rhabdomyosarcoma. In previous trials at this center, 60 (75%) of 80 patients had favorable responses to combinations of cyclophosphamide, doxorubicin, vincristine, and dactinomycin (5), while 18 (67%) of 27 patients with stage III or IV disease, treated in the Intergroup Rhabdomyosarcoma pilot study, responded favorably to an intensive induction regimen including vincristine, cyclophosphamide, dactinomycin, and radiation (6). Despite this similarity of overall response rates, none of our patients had complete responses, contrasted with 25% in the earlier St. Jude studies and 33% in the Intergroup Rhabdomyosarcoma study. One explanation for the lack of complete responses to doxorubicin-DTIC might be found in the large proportion of patients with extensive disease at diagnosis or unfavorable primary site (or both). Responses among patients with metastatic disease were inferior to those in a preceding St. Jude study that employed a four-drug regimen (seven of 12 patients vs 23 of 29). In four of the six patients with bone marrow replacement by tumor cells, there was clearing or a marked decrease of tumor infiltrate, but those improvements were short-lived.

Although DTIC combined with doxorubicin has been reported to be more effective and possibly more toxic than either drug alone in adults with soft tissue sarcoma (2), there are no reported studies in pediatric patients designed to confirm these data.

We conclude that DTIC combined with doxorubicin is active in childhood rhabdomyosarcoma, although its failure to induce complete responses makes it inferior to other commonly utilized induction regimens that employ combinations of vincristine, dactinomycin, cyclophosphamide, with or without doxorubicin.

REFERENCES

1. GREEN D, and JAFFE N. Progress and controversy in the treatment of childhood rhabdomyosarcoma. Cancer Treat Rev 5:7-27, 1978.
2. GOTTLIEB JA, BAKER LH, O'BRYAN RM, ET AL. Adriamycin (NSC-123127) used alone and in combination for soft tissue and bone sarcomas. Cancer Chemother Rep 6:271-282, 1975.
3. CANGIR A, MORGAN S, LAND V, ET AL. Combination chemotherapy with adriamycin (NSC-123127) and dimethyltriazeno imidazole carboxamide (DTIC) (NSC-45388) in children with metastatic solid tumors. Med Pediatr Oncol 2:183-190, 1976.
4. LUCE JK, THURMAN WG, ISAACS BL, ET AL. Clinical trials with the antitumor agent 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388). Cancer Chemother Rep 54:119-124, 1970.
5. ETCUBANAS E, and PRATT C. Chemotherapy as first choice treatment in rhabdomyosarcoma. *In* Proceedings of the XVII International Pediatric Congress, Nov 7-12, 1983, p 556.
6. RANEY B, GEHAN E, MAURER H, ET AL. Evaluation of intensified chemotherapy in children with advanced rhabdomyosarcoma (clinical groups III and IV). Cancer Clin Trials 2:19-28, 1979.

Treatment of Acute Leukemia With Amsacrine and High-Dose Cytarabine[1,2]

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We varied the number of doses of high-dose cytarabine (3 g/m²) given to patients with acute leukemia in relapse who were receiving therapeutic doses of amsacrine (600 mg/m²/course). Twelve patients received 13 courses of therapy. Two of six patients with acute nonlymphocytic leukemia and one of three with acute lymphoblastic leukemia achieved remission. Dose-limiting toxicity (diarrhea) was seen in patients receiving more than five daily doses of high-dose cytarabine. We conclude that therapeutic doses of amsacrine can be combined safely with five daily doses of high-dose cytarabine and that a formal study is now required to assess the efficacy of this regimen. [Cancer Treat Rep 69:1001–1002, 1985]

High-dose cytarabine (HiDAc), either alone or in combination with L-asparaginase, is effective therapy for acute nonlymphocytic leukemia (ANLL) (1–3). It has also been combined with amsacrine, and a high remission induction rate has been seen in ANLL in relapse (4). In the latter trial, maximum therapeutic doses of HiDAc were combined with a dose of amsacrine that is ineffective when used as a single agent, and it is possible that the high remission rate was due to the cytarabine alone (5). We have therefore treated patients with full therapeutic doses of amsacrine and varied the dose of HiDAc to define the optimal dose relationship for the combination.

METHODS

Patients with primary, refractory, and relapsed ANLL or acute lymphoblastic leukemia (ALL) and patients with blastic phase of chronic myelogenous leukemia (blCML) were eligible for treatment.

After giving informed consent patients received amsacrine at a dose of 200 mg/m² in 250 ml of 5% dextrose in water over 1 hour on 3 consecutive days. Cytarabine, 3 g/m², was diluted in 250–500 ml of 5% dextrose in water and infused over 3 hours. Cytarabine doses were given initially every 12 hours. The first patient received ten doses. Subsequent patients received 9, 8, 6, and 5 doses, with elimination of the 12-hour dose on Days 5, 4, 3 and 2, and 1, respectively. The final schedule for HiDAc was thus one dose daily for 5 days. The number of doses administered was reduced as unacceptable nonhematologic toxicity was encountered.

Complete remission was defined as the appearance of M1 bone marrow, a granulocyte count $> 1 \times 10^9$/L, and a platelet count $> 100 \times 10^9$/L.

RESULTS

A total of 12 patients were treated: six with ANLL, four with ALL, and two with blCML. The patients with ANLL included one who had cardiomyopathy and had received no prior chemotherapy, one in first relapse, two in second relapse, and two who had never achieved a prior remission. Two patients with ALL had failed primary induction chemotherapy, one was in first relapse, and one had a mediastinal lymphoma (positive for terminal deoxynucleotidyl transferase). Bone marrow was obtained at 7–10 days after chemotherapy to evaluate the effectiveness of the treatment in reducing leukemic cell infiltration.

Of the ten patients in whom bone marrow was assessed after chemotherapy, eight achieved aplasia and two achieved hypoplasia. In ANLL there was one remission with ten doses and one remission with five doses of HiDAc. There was one remission among the four patients with ALL; it occurred with five doses of HiDAc in the one patient in relapse.

The toxicity encountered is shown in table 1. All patients experienced nausea and vomiting. Stomatitis was

[1]Received Sept 10, 1984; revised Mar 13, 1985; accepted Mar 28, 1985.
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TABLE 1.—Toxicity with amsacrine-HiDAc

Toxic effect	No. of patient-courses*	
	> 5 HiDAc doses (n = 5)	5 HiDAc doses (n = 8)
Stomatitis	2	3
Diarrhea	4†	7
Hepatic‡	5	4
Conjunctivitis	1	0
Skin	1	0
Neurologic	0	1§

*1 patient received 2 courses.

†In 3 patients, > 5–10 L/day.

‡SGOT > 100 U/L; alkaline phosphatase > 200 U/L; and bilirubin > 4 mg/dl.

§Dysarthria and mild ataxia for 1 day.

mild and completely absent in many of the patients treated. Conjunctivitis, skin rash, and neurologic side effects occurred rarely. Hyperbilirubinemia (bilirubin > 4 mg/dl) was seen in four of five patients at the higher total doses of HiDAc and in only one of eight patients at the lower dose. No patient developed cardiac arrythmias. The most important nonhematologic toxic effect was diarrhea. This was virtually universal, but at more than five doses of HiDAc it was unacceptable in most patients.

DISCUSSION

An important principle of combination chemotherapy is that the regimen contains therapeutic doses of all its components. We therefore combined full therapeutic doses of amsacrine with varying doses of HiDAc in an effort to determine the maximum amount of HiDAc that could be safely given. In this series, the dose-limiting toxic effect was diarrhea. Although no patient experienced bowel perforation, the diarrhea that occurred in patients receiving more than five daily doses of HiDAc was considered unacceptable. Other nonhematologic toxicity was not dose-limiting, even with increased doses of HiDAc.

Since aplasia was seen in most of the patients treated with five daily doses of HiDAc, further escalation may not be required. This experience suggests that amsacrine at a dose of 200 mg/m^2 daily × 3 with cytarabine at a dose of 3 g/m^2 daily × 5 is a safe regimen which may prove useful in treating patients with acute leukemia. A formal study is now required to assess the efficacy of this regimen.

REFERENCES

1. RUDNICK S, CADMAN EC, CAPIZZI RL, ET AL. High dose cytosine arabinoside in refractory acute leukemia. Cancer 44:1189, 1979.
2. PREISLER HD, EARLY AP, RAZA A, ET AL. Therapy of secondary acute non-lymphocytic leukemia with cytarabine. N Engl J Med 308:21, 1983.
3. HERZIG RH, WOLFF SN, LAZARUS HM, ET AL. High dose cytarabine arabinoside therapy for refractory leukemia. Blood 62:361, 1983.
4. HINES JD, OKEN MM, MAZZA JJ, ET AL. High dose cytosine arabinoside and *m*-AMSA is effective therapy in relapsed acute nonlymphocytic leukemia. J Clin Oncol 2:549, 1984.
5. ARLIN ZA. Current status of amsacrine (AMSA) combination chemotherapy programs in acute leukemia. Cancer Treat Rep 67:967–970, 1983.

Aminoglutethimide-Induced Hematologic Toxicity: Worldwide Experience[1]

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Marked leukopenia and/or thrombocytopenia occurred in 12 of 1333 patients treated with aminoglutethimide (0.9% incidence). Depression of blood cell counts was evident within 7 weeks of starting treatment. No delayed toxicity was encountered. Blood cell counts recovered promptly upon cessation of aminoglutethimide in all but one patient, who died from septicemia and marrow aplasia. [Cancer Treat Rep 69:1003–1004, 1985]

Aminoglutethimide is receiving increased attention as a palliative agent in the treatment of breast and prostate cancer. Hematologic toxicity has occasionally been reported in patients treated with this aromatase inhibitor. Individual case reports of pancytopenia or thrombocytopenia are reported in the literature (1-3). The purpose of this report is to pool our collective experience to define the characteristics of the hematologic toxicity associated with aminoglutethimide therapy.

METHODS

Patients and Protocol

Each participating physician agreed to review the records of all patients treated with aminoglutethimide at his institution. Ninety percent of patients were treated for metastatic breast cancer and 10% were treated for metastatic prostate cancer. The starting dose of aminoglutethimide was 500 mg/day for 14 days. Thereafter, it was raised to 1000 mg/day. Almost all patients received concomitant steroid treatment, ie, hydrocortisone or medroxyprogesterone acetate. Patients were excluded if, in the opinion of the investigator, hematologic toxicity could possibly be ascribed to other concomitant medications or treatment. The investigator was asked to define the interval from starting therapy with aminoglutethimide to uncovering of hematologic toxicity, as well as the time to recovery of blood cell counts.

Severe hematologic toxicity was defined as a wbc count $< 1500/mm^3$ or a platelet count $< 50,000/mm^3$. These values were chosen because blood cell counts significantly below these levels would be expected to produce life-threatening toxicity.

RESULTS

There were 1333 patients treated with aminoglutethimide at the six participating institutions. Significant hematologic toxicity was reported in 12 patients, a 0.9% incidence (table 1). All but one institution reported patients with leukopenia and/or thrombocytopenia.

Seven patients developed only thrombocytopenia. Leukopenia was seen in two patients. Three patients had thrombocytopenia plus leukopenia (table 1). No pure rbc aplasia was reported. There was no reported relationship between hematologic and other aminoglutethimide toxicity (eg, fever, skin rash, CNS).

All hematologic toxicity was evident within 7 weeks of starting aminoglutethimide (table 2). No delayed toxicity was noted in these patients. The earliest reported depression of blood cell counts occurred 3 weeks after the initiation of treatment.

Recovery from depression of blood cell counts occurred in all but one patient by 3 weeks after discontinuation of medication. One patient, however, died of septicemia due to marrow aplasia. The bone marrow in this patient was taken from a nonirradiated area and showed no infiltration with tumor cells.

[1]Received Oct. 11, 1984; revised Feb 22, 1985; accepted Mar 21, 1985.

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TABLE 1.—Hematologic toxicity from aminoglutethimide*

Institution	No. of patients treated	No. of patients with hematologic toxicity
The Cancer Institute Peter MacCallum Hospital	580	2 (thrombocytopenia)
The Milton S. Hershey Medical Center	289	2 (thrombocytopenia) 1 (leukopenia) 2 (thrombocytopenia + leukopenia)
Cancer Control Agency of British Columbia	175	1 (thrombocytopenia)
M. D. Anderson Hospital and Tumor Institute	163	2 (thrombocytopenia) 1 (leukopenia)
Medizinische Klinik and Poliklinik der Universität	89	1 (thrombocytopenia + leukopenia)
Dana-Farber Cancer Institute	37	None
Total	1333	12

*Total incidence of hematologic toxicity = 0.9%.

TABLE 2.—Time to aminoglutethimide-induced hematologic toxicity and recovery

Patient No.	Hematologic toxicity (wks after starting aminoglutethimide)	Recovery (days after stopping aminoglutethimide)
	Thrombocytopenia	
1	7	7
2	5	3
3	3	5
4	7	11
5	4	14
6	6	14
7	7	21
	Leukopenia	
8	7	12
9	7	21
	Leukopenia + thrombocytopenia	
10	6	Died
11	4	5
12	6	14

It should be mentioned that at two institutions, cases of mild thrombocytopenia were reported (50,000-100,000 cells/mm^3). These cases are not recorded in this report due to our stringent definition of hematologic toxicity. All instances were discovered within the first 6 weeks of therapy, and the platelet counts recovered promptly within 7 days after discontinuation of aminoglutethimide.

DISCUSSION

We have reviewed the experience of 1333 patients treated with aminoglutethimide at six cooperating institutions. Marked depression of wbc and/or platelet counts occurred in 12 patients (0.9% incidence).

Hematologic toxicity from aminoglutethimide occurs within 3-7 weeks of starting the drug. Delayed toxicity was not observed in any patients. Based on this information, we would recommend performing a cbc and platelet count at Weeks 4, 8, and 12 after starting aminoglutethimide. Thereafter, we would suggest blood cell counts only as indicated by clinical symptoms. In all but one case, the encountered toxicity was readily reversible upon cessation of treatment.

The question remains as to the mechanism of leukopenia and/or thrombocytopenia induced by aminoglutethimide. The absence of patients with bone marrow toxicity within 3 weeks of initiation of drug treatment would support the concept of an immunologically mediated injury (1). An alternative explanation is that the dose of aminoglutethimide is important. In most patients in this retrospective review, the aminoglutethimide dose was 500 mg/day for the first 14 days. Thereafter, this dose was raised to 1000 mg/day. It will be most instructive to see if hematologic toxicity is encountered as lower doses are administered. A lower dose of aminoglutethimide can inhibit aromatization and appears to be equivalent to high-dose treatment with regard to remission induction.

REFERENCES

1. LAWRENCE B, SANTEN RJ, LIPTON A, ET AL. Pancytopenia induced by aminoglutethimide in the treatment of breast cancer. Cancer Treat Rep 62:1581-1583, 1978.
2. ASHBURY RF, BAKEMEIER RF, FOLSCH E, ET AL. Treatment of metastatic breast cancer with aminoglutethimide. Cancer 47: 1954-1958, 1981.
3. ARDMAN B, and RUDDERS R. Aminoglutethimide-induced thrombocytopenia. Cancer Treat Rep 66:1785-1786, 1982.

Brachial Plexus Neuropathy Following High-Dose Cytarabine in Acute Monoblastic Leukemia[1,2]

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We describe brachial plexus neuropathy with high-dose cytarabine (Ara-C) therapy in a man who had acute monoblastic leukemia. Signs and symptoms of brachial plexus neuropathy appeared on two occasions within hours of exposure to high-dose Ara-C. Central nervous system complications have been described following systemic and intrathecal Ara-C. High-dose Ara-C has not been implicated previously as a cause of brachial plexus neuropathy. [Cancer Treat Rep 69:1005–1006, 1985]

Cytosine arabinoside (Ara-C) is a chemotherapeutic agent widely used in the treatment of acute nonlymphocytic leukemia. Peripheral sensory neuropathy (1) and confusion (2) are complications reported in association with conventional-dose Ara-C therapy. An increased number of neurological complications have recently been reported, however, with the advent of high-dose Ara-C (1–3 g/m^2) therapy for patients with leukemia who are refractory to conventional doses. High-dose systemic Ara-C is associated with cerebellar dysfunction (3), somnolence, confusion, headache, and seizures (4). We now describe brachial plexus neuropathy (BPN) associated with high-dose systemic Ara-C administration in a man with acute monoblastic leukemia.

CASE REPORT

A 47-year-old white man, previously well, was admitted on February 7, 1984, for evaluation of fever and fatigue for 1 month. He had no history of antecedent viral symptoms or immunizations. The general and neurological examinations on admission were normal. Laboratory data on admission, including cerebrospinal fluid, were normal except for a wbc count of 17,600/mm^3 (69% blasts, 4% segmented neutrophils, 26% lymphocytes, 3% immature myeloid cells, and 2% eosinophils). Bone marrow examination revealed a cellularity of 90% blasts. Myeloid to erythroid ratio was > 25:1. Auer rods were absent. Special stains were negative, with the exception of butyrate esterase, which was strongly positive. A diagnosis of acute monoblastic leukemia was made.

Initial induction chemotherapy consisted of daunorubicin (45 mg/m^2 daily for three doses) and Ara-C (100 mg/m^2 daily for seven doses). The patient failed to respond to a second induction course. On April 4, 1984, high-dose Ara-C was begun at 3 g/m^2 (6.3 g) over 3 hours every 12 hours times four doses and was well-tolerated. On April 11, 1984, a second course of high-dose Ara-C was begun, and 6 hours after the start of the first infusion into the right subclavian vein, the patient noted the abrupt onset of left arm and shoulder pain. This was followed in 12 hours by right shoulder and arm pain. There was associated weakness of the left arm proximally, particularly with attempted abduction and extension. Sensory symptoms and bowel and bladder dysfunction were absent. Neurological examination on April 12 was normal except for the motor examination of the upper extremities. Abduction, adduction, and extension of the left arm were moderately weak. Initiation of abduction of the left arm was profoundly weak. Extension of the elbow was mildly weak. On the right, there was moderate weakness of initiation of abduction and mild weakness in extension and abduction of the arm. He received all four doses of his second course of high-dose Ara-C followed by improvement of

[1]Received Oct 11, 1984; revised Mar 15, 1985; accepted Mar 19, 1985.

[2]The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

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his pain and weakness over several days. On April 19, a third course of high-dose Ara-C was given and within 2 hours of the start of the first infusion he experienced severe exacerbation of bilateral shoulder pain and weakness in both upper extremities. Neurological examination at that time revealed worsening of his weakness in abduction, adduction, and extension of the left arm and winging of the right scapula, with increased weakness of extension of the right arm and elbow. Moderate atrophy of the left supraspinatus and infraspinatus muscles was noted. A metrizimide myelogram and computerized tomography scan through C5-C8 with computerized tomographic cuts through the brachial plexus were normal. On May 2, electromyography was consistent with denervation in muscles supplied by the upper and middle trunks of the brachial plexus on the left and upper trunk on the right. Somatosensory evoked responses revealed an absent Erb's response on the left with corresponding delay over the contralateral motor strip. Because of the side effects, his last dose of Ara-C was not given. His symptoms and signs of BPN did not change over the ensuing month despite relapse of his leukemia. On June 10, the patient developed central nervous system leukemia and died several days later. An autopsy was not performed.

DISCUSSION

BPN is a syndrome characterized by acute pain over the shoulder followed by weakness and atrophy in the muscles of the arm (5). The weakness may be proximal, distal, or both, and many cases arise unilaterally. BPN is associated with numerous causes, including diabetes mellitus, polyarteritis nodosa, immunizations, and other systemic diseases of ill-defined origin (6). Plexitis following iv heroin injection (7), insect stings (8), and jellyfish stings (9) is also reported.

Signs and symptoms of BPN are also seen in patients with cancer (10). Tumor infiltration and radiation injury are the two most common etiologies (11,12). Less commonly, brachial plexus dysfunction is caused by trauma during surgery (13) or radiation-induced tumors (14). Paralytic brachial neuritis is reported in Hodgkin's disease (15) and rarely in other cancers (5).

BPN has not previously been described as a complication of Arà-C therapy. The association of our patient's BPN with Ara-C is supported by the temporal relationship between infusion of Ara-C and the onset of his BPN as well as the occurrence of symptoms following re-exposure to the drug. The absence of any evidence of the known causes of BPN also suggests that his illness was drug-induced.

The mechanism for Ara-C-induced BPN is unclear. One hypothesis is a direct toxic effect. Parenteral injections of substances, most notably heavy metals, other toxins, and various sera, may produce a spectrum of neuritides, including plexitis (16,17). Challenor et al (7) state that these neuritides occur remote from the site of injection, as in our patient, and all patients have a predilection for radial mononeuropathy and brachial plexitis. An immune mechanism is also implicated with the first occurrence of BPN 1 week after an asymptomatic exposure to high-dose Ara-C. Rassiga et al (18) reported a case of Ara-C-induced anaphylaxis, gave evidence to support an allergic reaction to Ara-C, and successfully carried out desensitization. The possibility of tumor infiltration into the brachial plexus in our patient is not likely because his neurological findings were unchanged after 1 month despite relapse of his leukemia.

We feel that this complication was significant in that it changed the course of this patient's treatment. Use of high-dose Ara-C is relatively new and patients receiving this regimen should be monitored carefully for signs of peripheral nervous system toxicity.

REFERENCES

1. RUSSELL JA, and POWLES RL. Neuropathy due to cytosine arabinoside. Br Med J 4:652-653, 1974.
2. SHAH SS, RYBAK ME, and GRIFFIN TW. The cytarabine syndrome in an adult. Cancer Treat Rep 67:405-406, 1983.
3. LAZARUS HM, HERZIG RH, HERZIG GP, ET AL. Central nervous system toxicity of high-dose systemic cytosine arabinoside. Cancer 48:2577-2582, 1981.
4. RUDNICK SA, CADMAN EC, CAPIZZI RL, ET AL. High dose cytosine arabinoside (HDARAC) in refractory acute leukemia. Cancer 44:1189-1193, 1979.
5. TSAIRIS P, DYCK PJ, and MULDER DW. Natural history of brachial plexus neuropathy. Arch Neurol 27:109-117, 1972.
6. TSAIRIS P. Brachial plexus neuropathies. *In* Peripheral Neuropathy (Dyck PJ, Thomas PK, and Lambert E, eds). Philadelphia, W. B. Saunders Co, 1975, vol 1, pp 659-767.
7. CHALLENOR YB, RICHTER RW, BRUUN B, ET AL. Nontraumatic plexitis and heroin addiction. JAMA 225:958-961, 1973.
8. GOLDSTEIN NP, RUCKER CW, and WOLLMAN HW. Neuritis occurring after insect stings. JAMA 173:1727-1730, 1960.
9. FILLING-KATZ MR. Mononeuritis multiplex following jellyfish stings. Ann Neurol 15:213, 1984.
10. POSNER JB. Neurological complications of systemic cancer. Med Clin N Am 63:783-800, 1979.
11. KORI SH, FOLEY KM, and POSNER JB. Brachial plexus lesions in patients with cancer: 100 cases. Neurology 31:45-50, 1981.
12. CASCINO TL, KORI S, KROL G, ET AL. CT of the brachial plexus in patients with cancer. Neurology 33:1553-1557, 1983.
13. JACKSON L, and KEBTS AS. Mechanisms of brachial plexus palsy following anesthesia. Anesthesiology 26:190-194, 1965.
14. FOLEY KM, WOODRUFF JM, ELLIS FT, ET AL. Radiation-induced malignant and atypical peripheral nerve sheath tumors. Ann Neurol 7:288-296, 1980.
15. PEZZIMENTI JF, BRUCKNER HW, and DECONTI RC. Paralytic brachial neuritis in Hodgkin's disease. Cancer 31:626-629, 1973.
16. GATHIER JC, and BRUYN GW. The vaccinogenic peripheral neuropathies. *In* Handbook of Clinical Neurology (Vinken PJ, and Bruyn GW, eds). Amsterdam, North-Holland Publishing Co, 1970, vol 8, pp 86-94.
17. GATHIER JC, and BRUYN GW. The serogenetic peripheral neuropathies. *In* Handbook of Clinical Neurology (Vinken PJ, and Bruyn GW, eds). Amsterdam, North-Holland Publishing Co, 1970, vol 8, pp 95-111.
18. RASSIGA AL, SCHWARTZ HJ, FORMAN WB, ET AL. Cytarabine-induced anaphylaxis: demonstration of antibody and successful desensitization. Arch Intern Med 140:425-426, 1980.

Intensive Induction Treatment of Small Cell Bronchogenic Carcinoma With Cyclophosphamide, Methotrexate, and Etoposide[1,2]

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Fifty-six patients with histologically confirmed small cell bronchogenic carcinoma were treated with cyclophosphamide, methotrexate, and etoposide. While methotrexate doses were modified for mucositis during the 6-week induction period, none of the drug doses were modified for hematologic toxicity. The overall response rate was 66%, with 16% complete remissions; median survival duration was 28 weeks. In 12 patients, the leukocyte count fell below 1000/mm^3, and there were four deaths in febrile, leukopenic patients. Diffuse pulmonary infiltrates were observed in eight patients, and three died from respiratory insufficiency. Lung biopsy in two patients and autopsy in two additional patients showed interstitial changes consistent with drug injury. This regimen produced considerable hematologic and apparent pulmonary toxicity while offering no advantage in response rate or survival duration. [Cancer Treat Rep 69:1007–1009, 1985]

Collective data from multiple phase II trials during the 1970s showed that small cell bronchogenic carcinoma (SCBC) is relatively sensitive to combination chemotherapy regimens (1). In a randomized trial comparing "low-dose" versus "intensive" induction treatment consisting of cyclophosphamide, lomustine, and methotrexate (CCM), Cohen et al (2) observed a higher response rate and a longer median survival in the more intensively treated patients.

Virtually simultaneous with these developments, a report of phase II trials of etoposide suggested that it was one of the most active drugs in SCBC (3). The purpose of our study was to test the concept of intensive induction treatment using a modified version of the regimen of Cohen et al (2). Etoposide was substituted for lomustine because of apparent greater activity [50% response rate for etoposide (3) vs 13% for lomustine (4)].

MATERIALS AND METHODS

Patients with SCBC, which was confirmed histologically or by cytologic specimens obtained at bronchoscopy, were eligible for this study. Other eligibility requirements included: leukocyte count > 4000/mm^3, platelet count > 100,000/mm^3, BUN < 25 mg/dl, serum creatinine < 1.5 mg/dl, age < 70 years, Eastern Cooperative Oncology Group (ECOG) performance status < 3, the absence of brain metastases, and an informed written consent.

Drug treatment consisted of cyclophosphamide (700 mg/m^2 iv on Days 1 and 22), etoposide (60 mg/m^2 iv on Days 1–5 and on Days 22–26), and methotrexate (15 mg/m^2 orally on Days 8, 11, 15, 18, 29, 32, 36, and 39 during the first 42 days of treatment). During the induction period, drug doses were not modified for leukopenia or thrombocytopenia unless there was evidence of infection or bleeding. Methotrexate doses were modified for mucositis. During maintenance treatment, cyclophosphamide and methotrexate were given on the same schedule, but the doses were reduced to 500 mg/m^2 and 10 mg/m^2, respectively. The same dose of etoposide was given on Days 1, 3, and 5. Treatment was continued until tumor progression was observed or for 1 year in patients in whom complete remission was achieved. Toxicity and tumor responses were defined according to ECOG criteria (5).

[1]Received Oct 19, 1984; revised Feb 15, 1985; accepted Feb 19, 1985.

[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in this study: Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (*P. Bonomi*, *W. O'Reilly*, and *N. Bunting*, CA-10948); Albert Einstein College of Medicine, Bronx, NY (*S. E. Vogl*, CA-14958); Albany Medical College, NY (*J. C. Ruckdeschel*, CA-06594); University of Massachusetts, Boston (*L. Stolbach*); and American Oncologic Hospital, Philadelphia, PA (*R. H. Creech*, CA-18281).

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RESULTS

From October 1980 to January 1982, 56 patients were entered in this trial. All patients were evaluable for response, survival, and toxicity.

Patient characteristics are described in table 1. The overall response rate was 66%, with 16% of the responses being classified as complete. Response rates and median survival durations for the entire group and for the subgroups of patients with limited or extensive disease are shown in table 2.

The lowest leukocyte count occurred during induction treatment in 55 of 56 patients, with a median leukocyte count nadir of 1900/mm^3. Four febrile patients died from apparent sepsis when their leukocyte counts were < 1000/mm^3. The median platelet count nadir was 153,000/mm^3. No episodes of bleeding secondary to thrombocytopenia were observed. Nausea and vomiting as well as alopecia were observed in most patients. Stomatitis occurred in 24 patients (43%).

Bilateral pulmonary infiltrates were observed in eight patients from 7 to 14 weeks after starting treatment. Transbronchial lung biopsies were performed in two patients. They revealed interstitial mononuclear infiltrate with negative cultures and no evidence of *Pneumocystis carinii*. Autopsies done on two other patients revealed interstitial pulmonary fibrosis. Three of these patients died from pulmonary insufficiency. An additional patient developed interstitial pulmonary infiltrates which proved to be due to *P. carinii*.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Median age in yrs: 63	
Males/females	37/19
Performance status 0-1/2	40/16
Limited/extensive disease	17/39

DISCUSSION

Laboratory data and theoretical considerations suggest that higher doses of chemotherapy produce a greater degree of tumor regression (6). This concept was tested in SCBC in a small randomized trial in which a three-drug combination chemotherapy regimen unmodified for hematologic toxicity was compared to lower doses of the same three drugs with dose modifications for myelosuppression. The response rate and median survival for the high-dose regimen were 96% and 10.5 months, respectively, compared to 45% and 5 months for the low-dose regimen (2). Based on these preliminary observations, ECOG designed a trial in which "intensive induction" treatment including cyclophosphamide, lomustine, and methotrexate (CCM) without dose modifications during the 6-week induction period was compared to the "standard induction" regimen which was identical except for a 50% lower dose of cyclophosphamide and for the fact that drug doses were modified for toxicity in the "standard" treatment.

Assuming that the more intensive therapy would be more effective, the cyclophosphamide, methotrexate, and etoposide (CMVP16) regimen was designed as a possible treatment for a future randomized study. The overall response rate of 66% observed with this regimen is similar to the 64% for intensive CCM and 54% for standard CCM ($p = 0.04$ for standard vs intensive CCM) (7). The median survival durations for patients treated with intensive CCM or standard CCM were 41 and 36 weeks, respectively ($p = 0.04$) (6). The 28-week median survival CMVP16 suggests that this regimen may be inferior to intensive and standard CCM.

The modest improvement in survival observed with intensive versus standard CCM must be weighed against the 53% life-threatening and 7% lethal toxicity with intensive CCM compared to the 7% life-threatening and 1% lethal toxicity with standard CCM. Similarly, CMVP16 was associated with 7% lethal toxicity secondary to leukopenia as well as 5% lethal pulmonary toxicity, which has been described in detail by Zimmerman et al (8).

In conclusion, induction treatment with CMVP16 unmodified for hematologic toxicity has resulted in considerable hematologic and pulmonary toxicity in patients with SCBC, without producing a higher complete remission rate or a longer median survival than less toxic regimens (1). Moreover, our results and those of Vogl and Mehta (7) raise a question about the value of intensive induction therapy in SCBC.

TABLE 2.—Response

	No. of evaluable patients	No. of patients (%) achieving		Overall response (%)	Median survival (wks)
		CR	PR		
Limited disease	17	5 (29)	5 (29)	58	40
Extensive disease	39	4 (10)	23 (60)	70	23.5
All patients	56	9 (16)	28 (50)	66	28

REFERENCES

1. BUNN PA, COHEN H, IHDE DC, ET AL. Advances in small cell bronchogenic carcinoma. Cancer Treat Rep 61:333-342, 1977.
2. COHEN MH, CREAVEN PJ, FOSSIECK BE, ET AL. Intensive chemotherapy of small cell bronchogenic carcinoma. Cancer Treat Rep 61:349-354, 1977.
3. HANSEN M, HIRSCH F, DOMBERNOWSKY P, ET AL. Treatment of small cell anaplastic carcinoma of the lung with VP-16-213 (NSC141540), 4'-demethylepipodophyllotoxin 9-(4,6-O-ethylidene-β-D-glucopyranoside). Cancer 40:633-637, 1977.
4. BRODER LE, COHEN MH, and SELAWRY DS. Treatment of bronchogenic carcinoma: II small cell. Cancer Treat Rev 4:219-260, 1977.
5. OKEN MM, CREECH RH, TORMEY DC, ET AL. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.
6. FREI E, III, and CANELLOS G. Dose: a critical factor in cancer chemotherapy. Am J Med 69:585-594, 1980.
7. VOGL SE, and MEHTA C. High-dose cyclophosphamide in the induction chemotherapy of small cell lung cancer—minor improvements in the rate of remission and in survival. Abstracts: The III World Conference on Lung Cancer, 1982, p 168.
8. ZIMMERMAN MS, RUCKDESCHEL JC, and HUSSAIN M. Chemotherapy induced interstitial pneumonitis during treatment of small cell anaplastic lung cancer. J Clin Oncol 2:396-405, 1984.

Deep Venous Thrombosis During Therapy for Hodgkin's Disease[1]

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Upon reviewing the case records of 177 patients with Hodgkin's disease, we identified ten patients (6%) with deep vein thromboses confirmed by diagnostic tests. Most of the patients initially presented with advanced Hodgkin's disease as defined by stage and constitutional symptoms. Thrombotic episodes usually occurred between cycles of chemotherapy in the absence of clinically detectable tumor. Infusion of chemotherapeutic vesicants may have contributed to the high proportion of upper extremity deep venous thrombosis in this series. Thrombotic episodes did not necessarily imply recurrent disease. Three patients developed thromboses after completion of therapy and remained free of Hodgkin's disease or other malignancies at 85+, 18+, and 17+ months of follow-up. [Cancer Treat Rep 69:1011–1013, 1985]

Armand Trousseau (1) was the first to describe the association of thrombotic episodes with an underlying malignancy. Prior to the use of chemotherapy, thrombotic complications were rarely reported with Hodgkin's disease (2,3). In one series, deep vein thromboses for patients undergoing laparotomy and splenectomy for the staging of Hodgkin's disease were no more frequent than for a control group undergoing elective cholecystectomy (4). In a retrospective review of 177 case records at the National Cancer Institute, we identified ten patients (6%) with Hodgkin's disease who required parenteral anticoagulation for documented deep venous thrombotic episodes. Half of these patients presented with clots localized to the subclavian or axillary vein. Chemotherapeutic vesicants may be responsible for the unexpected frequency of thrombotic episodes.

PATIENTS AND METHODS

We reviewed the treatment records of 177 consecutive patients with a diagnosis of Hodgkin's disease admitted to the Medicine Branch of the National Cancer Institute between January 1, 1974, and March 1, 1983. We identified ten patients with deep venous thrombotic episodes confirmed by diagnostic tests. Two patients with unilateral swelling of the lower extremity were excluded because of lack of documentation. We also omitted several episodes of superficial thrombophlebitis at sites of chemotherapeutic administration. We examined the ten case records for the following variables: age, sex, stage, histology, site of thrombosis, diagnostic procedures, treatment of thrombosis, routine coagulation tests, current disease status, and the timing of thrombosis in relation to tumor therapy.

Venography of the affected extremity was considered positive with consistent intraluminal filling defects or interruption of venous filling in eight cases. Scanning with ^{125}I-labeled fibrinogen was employed in one case with a subclavian vein thrombosis. In one case, a positive ventilation-perfusion scan was reported in the clinical setting of unilateral leg swelling, dyspnea, and pleuritic chest pain. The perfusion scan utilized ^{99}Tc-labeled macroaggregated albumin, while the ventilation scan employed ^{133}Xe radioisotope with wash-in and wash-out studies.

RESULTS

Significant thrombotic episodes were documented in ten patients with Hodgkin's disease in this series, representing almost 6% of the 177 evaluable case records (table 1). Eight of the ten patients were female, ranging in age from 18 to 37 years; the two males were 46 and 54 years old. Nodular sclerosis was the most frequent histologic subtype (seven of ten patients), and stage III or IV disease accounted for seven of the cases. Constitutional symptoms, designated "B" in the staging system, were present in seven patients. We treated all ten patients

[1]Received Jan 2, 1985; revised Feb 21, 1985; accepted Feb 27, 1985.

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TABLE 1.—Hodgkin's disease patients with thrombotic episodes*

Patient No.	Age (yrs)/ sex	Histology	Stage/ involved areas	Thrombotic episode	Diagnostic tests	Timing of thrombotic episode	Treatment	Disease status
1	13/F	MC	IIIB/left hilar mass, para-aortic adenopathy	Right axillary vein	Venography	13 mos after chemotherapy & radiotherapy	Heparin infusion × 7 days, warfarin × 6 mos	Free of disease 7 yrs after thrombosis
2	46/M	MC	IIIB/bilateral hilar & para-aortic adenopathy	Right axillary vein	Venography	During chemotherapy (after Cycle 3)	Heparin infusion × 7 days, warfarin × 6 mos	Free of disease 5 yrs after thrombosis
3	35/F	NS	IVB/right upper lobe nodules	Right subclavian vein	Radionuclide scan	During chemotherapy (after Cycle 2)	Heparin infusion × 10 days	Deceased with progressive disease 14 mos after DVT
4	26/F	MC	IIA/cervical & axillary adenopathy	Left femoral vein	Venography	During chemotherapy (after Cycle 1)	Heparin infusion × 7 days, warfarin × 3 mos	Free of disease 4 yrs after thrombosis
5	17/F	NS	$IIIA_1S$/low-bulk mediastinal & spleen	Right leg swelling & symptoms of PE	Ventilation-perfusion scan	During chemotherapy (after Cycle 4)	Heparin infusion × 7 days, warfarin × 6 mos	Free of disease 4 yrs after thrombosis
6	18/F	NS	IIB/massive mediastinal	Right axillary vein; left axillary vein	Venography; venography	During chemotherapy (after Cycle 9); 18 mos after chemotherapy & radiotherapy	Heparin infusion × 10 days; heparin infusion × 7 days	Free of disease 3 yrs after first DVT; 1½ yrs after second DVT
7	37/F	NS	IIB/low-bulk mediastinal & supraclavicular adenopathy	Left femoral vein	Venography	During salvage chemotherapy (after Cycle 1) following prior chemotherapy & radiotherapy	Heparin infusion × 10 days, warfarin and caval umbrella	Progressive disease
8	54/M	NS	IIIB/para-aortic & hilar	Left femoral vein × 3; PE	Venography; ventilation-perfusion scan	Before and during chemotherapy (after Cycles 6 & 8)	Heparin infusion × 8 days, warfarin × 8 mos	Progressive disease
9	28/F	NS	IIIB/left cervical & spleen	Left axillary vein	Venography	During chemotherapy (after Cycle 3)	Heparin infusion × 7 days, warfarin × 6 wks	Free of disease 3 yrs after DVT
10	32/F	NS	IIIAE/massive mediastinal	Left femoral vein	Venography	1 mo after chemotherapy & radiotherapy	Heparin infusion × 8 days, warfarin × 6 mos	Free of disease 1½ yrs after DVT

*MC = mixed cellularity, NS = nodular sclerosing, DVT = deep vein thrombosis, and PE = pulmonary embolus.

with MOPP chemotherapy (mechlorethamine, vincristine, procarbazine, and prednisone). Two patients received alternating cycles of MOPP and ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine), and one received alternating cycles of MOPP and SCAB (streptozocin, lomustine, doxorubicin, and bleomycin).

The thrombotic episode involved the upper extremity in five patients and the lower extremity in the other five. The thrombosis occurred between cycles of chemotherapy in seven of the ten patients. At the time of the clotting episode, no tumor was detectable in six of these patients. One patient had a left femoral vein thrombosis prior to chemotherapy, with recurrence after Cycles 6 and 8 despite adequate warfarin sodium anticoagulation. As both arms were employed alternately for chemotherapy administration, upper extremity thromboses always occurred in limbs exposed to vesicants. Of four patients who received mantle irradiation prior to the thrombotic episode, two developed clots in the upper extremity and two thrombosed in the lower extremity.

Three patients had thrombotic episodes following completion of all tumor therapy. In two of these three cases, the thrombosis occurred after > 1 year had elapsed from completion of treatment. Thorough re-evaluation failed to disclose disease recurrence. All three patients with thrombosis following tumor therapy were still free of disease at the time of this review, with 85+, 18+, and 17+ months of follow-up after the time of thrombosis. Two

patients were using birth control pills prior to the clotting episode and one woman was markedly obese. No other predisposing factors for thrombosis were evident, such as hypertension, liver dysfunction, diabetes mellitus, or decreased ambulation.

The development of collateral circulation with prompt resolution of the edema was the rule for all five patients with clots of the upper extremity. Two patients had postphlebitic symptoms in the affected leg. All thrombotic episodes were treated with heparin by iv infusion over 7-10 days. Warfarin therapy ranged from 6 weeks to 8 months, but was not administered to two patients with upper extremity thrombosis. At the time of this review, seven patients were alive and free of disease, two were alive with relapse of disease, and one had died with progression of tumor.

DISCUSSION

We identified ten patients with documented deep venous thromboses in this retrospective review. Most of these patients had advanced Hodgkin's disease as defined by stage and by constitutional "B" symptoms. Three patients developed thromboses after the completion of tumor therapy, two of these three after a 1-year hiatus. Despite thorough re-evaluations and frequent follow-ups, all three patients have remained free of disease. Idiopathic thrombosis did not herald a relapse of Hodgkin's disease or a second malignancy in these cases.

The various causes of deep vein thrombosis are categorized into three basic mechanisms: impairment of blood flow, vascular disease or injury, or a hypercoagulable state. All patients except one had chemotherapy prior to their first reported clotting episode. The unexpectedly high proportion of upper extremity thrombosis (50% of patients) suggests possible chemical injury to the vascular system. Increased thrombotic episodes have been reported during chemotherapy for prostate cancer (5) and for breast cancer (6,7). Weiss et al (6) noted 24 of 433 (5%) episodes of venous thromboses during postmastectomy chemotherapy without concurrent evidence of tumor recurrence. Goodnough et al (7) reported a higher thrombotic rate of 17.6% in 159 patients treated with combination chemotherapy for stage IV metastatic breast carcinoma. Unfortunately, there are no comparative data on the incidence of deep vein thromboses in untreated Hodgkin's disease or after radiotherapy alone. This report is the first to describe deep vein thromboses following chemotherapy for Hodgkin's disease. Significant obesity with chronic venous stasis may have predisposed one patient to a femoral vein thrombosis. Two patients were taking birth control pills prior to upper extremity thromboses; although thrombophlebitis occurs more frequently in the setting of exogenous estrogen, axillary vein involvement represents a rather unusual presentation. Routine coagulation tests did not suggest chronic disseminated intravascular coagulation as an etiology for the thromboses in our series. Tests for protein C, protein S, or antithrombin III deficiency were not performed.

There were six episodes of upper extremity thrombosis among five of the patients in this series. Spontaneous clotting of the axillary or subclavian veins historically accounted for < 2% of all deep vein thromboses (8). However, this percentage is undoubtedly higher today given the ubiquitous use of central venous catheters. Patients classically present with unilateral arm swelling, dusky discoloration, dilated superficial collateral veins, and aching after exertion. Immediate heparin anticoagulation is recommended by most authors, since subsequent pulmonary emboli were reported most frequently in patients with underlying malignancies (8-10). Mediastinal involvement, mantle irradiation, and administration of chemotherapeutic vesicants may all contribute to the unexpectedly high rate of upper extremity thrombosis in this series. Prior chemotherapy was the only variable common to all six episodes of axillary or subclavian vein thrombosis.

REFERENCES

1. TROUSSEAU A. Phlegmasia alba dolens. Lectures on Clinical Medicine, delivered at the Hotel-Dieu, Paris. London, New Sydenham Society, 1872, vol 5.
2. ACKERMAN RF, and ESTES JE. Prognosis in idiopathic thrombophlebitis. Ann Intern Med 34:902-910, 1951.
3. LIEBERMAN JS, BORRERO J, URDANETA E, ET AL. Thrombophlebitis and cancer. JAMA 177:542-545, 1961.
4. DAWSON AA, BENNET B, JONES PF, ET AL. Thrombotic risks of staging laparotomy with splenectomy in Hodgkin's disease. Br J Surg 68:842-845, 1981.
5. KASIMIS BS, and SPIERS ASD. Thrombotic complications in patients with advanced prostatic cancer treated with chemotherapy. Lancet 1:159, 1979.
6. WEISS RB, TORMEY DC, HOLLAND JF, ET AL. Venous thrombosis during multimodal treatment of primary breast carcinoma. Cancer Treat Rep 65:677-679, 1981.
7. GOODNOUGH LT, SAITO H, MANNI A, ET AL. Increased incidence of thromboembolism in stage IV breast cancer patients treated with a five-drug chemotherapy regimen. Cancer 54:1264-1268, 1984.
8. COON WW, and WILLIS PW. Thrombosis of axillary and subclavian veins. Arch Surg 94:657-663, 1967.
9. CAMPBELL CB, CHANDLER JG, TEGTMEYER CJ, ET AL. Axillary, subclavian, and brachiocephalic vein obstruction. Surgery 82:816-826, 1977.
10. PRESCOTT SM, and TIKOFF G. Deep vein thrombosis of the upper extremity: a reappraisal. Circulation 59:350-355, 1979.

Alternating Combination Chemotherapy Using Cisplatin-Bleomycin With Sequential Methotrexate-5-FU Plus Leucovorin Rescue in Patients With Head and Neck Cancer[1]

L. Panasci,* D. Gravenor, M. Black, S. Frenkiel, and R. Margolese[2,3]

Thirty-two patients (only two had previously untreated disease) with squamous cell carcinoma of the head and neck were treated with alternating combination chemotherapy. The regimen consisted of cisplatin (50 mg/m^2) and bleomycin (30 units) given iv on Days 1 and 2, alternated with 1 hour of sequential methotrexate (200 mg/m^2) and 5-FU (600 mg/m^2) given iv, plus oral leucovorin rescue on Day 22. The entire cycle was repeated every 5 weeks (less than or equal to five cycles). Objective tumor regression was obtained in 33% of the 30 evaluable patients, with 13% complete regression. The median duration of response was only 3 months. Evidence of response occurred within one cycle, and was maximal after two cycles. Toxicity was very mild. Alternating combination chemotherapy is not more effective than either combination chemotherapy alone. [Cancer Treat Rep 69:1015–1017, 1985]

Methotrexate (MTX) is effective in the treatment of recurrent squamous cell carcinoma of the head and neck (SCHN) following surgery and/or radiotherapy, with reported response rates as high as 50% and a median duration of response generally < 4 months (1). Sequential MTX-5-FU with leucovorin rescue has resulted in an overall response rate of approximately 60% (approximately 10%–20% complete responses), with a median duration of response of < 4 months in patients with recurrent SCHN (2,3). The treatment of recurrent SCHN with cisplatin, bleomycin, and vincristine results in response rates of approximately 45% (10% complete responses). Responses with cisplatin and bleomycin have been seen in patients who have failed prior MTX chemotherapy (4,5).

The purpose of this study was to determine if alternating two effective combination chemotherapies in patients with SCHN would: (*a*) increase the overall response rate; (*b*) increase the complete response rate; and/or (*c*) increase the duration of response.

MATERIALS AND METHODS

All patients with histologically documented SCHN were eligible for this study. Patients had recurrent tumor or stage III or IV disease. Performance status was determined by the Eastern Cooperative Oncology Group (ECOG) scale from 0 to 4. Patients were required to have serum creatinine ⩽ 1.5 mg/dl, a wbc count ⩾ 4000/μl, and a platelet count ⩾ 150,000/μl.

Patients received cisplatin (50 mg/m^2) and bleomycin (30 units iv) on Days 1 and 2. The cisplatin was administered in the hospital with vigorous hydration (at least 4 L of iv fluids daily) and furosemide. Bleomycin was infused over 30 minutes in the prehydration period. On Day 22, the patient received MTX (200 mg/m^2 iv bolus) followed 1 hour later by 5-FU (600 mg/m^2 iv bolus) in the outpatient department without hydration. Twenty-four hours later, leucovorin (20 mg orally every 6 hours × six doses) was begun. The drugs were recycled every 35 days. Samples of peripheral blood were routinely obtained on Days 1 and 22 of each cycle to assess hematological, renal, and hepatic functions. After five cycles of this alternating chemotherapy, patients who continued to respond received sequential MTX-5-FU with leucovorin as outlined above every 2-3 weeks until relapse or for 5 months. After 1 year of treatment, all chemotherapy was stopped.

Partial response was defined as a ⩾ 50% reduction in the sum of the products of the greatest perpendicular

[1]Received Nov 19, 1984; revised Feb 11, 1985; accepted Feb 14, 1985.

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[3]We thank Dr. R. Sunnenblick for help in data accumulation and Sarah Scholl for typing the manuscript.

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diameters of all measurable lesions. Complete response was defined as disappearance of all clinically detectable disease. The patients were assessed for response by the medical oncologists (L. Panasci and D. Gravenor) and the surgeon (M. Black); there was full agreement among the physicians concerning the assessment of response. The duration of response for patients with recurrent disease was the time from the achievement of a response to progression of disease. Survival was the period of time from the start of chemotherapy to death.

RESULTS

Thirty-two patients consented to participate in the study. One patient was ineligible because of the presence of two separate malignancies (SCHN and biopsy-proven small cell carcinoma of the lung), and another was ineligible because of nonmeasurable disease. Thirty patients (25 males and five females, with a median age of 59 years) were evaluable for response to chemotherapy. Only two patients had not been previously treated with surgery or radiotherapy. Three patients had received previous chemotherapy (bleomycin in two patients, and cyclophosphamide and doxorubicin plus vincristine in one).

Ten of 30 patients responded to the alternating combination chemotherapy, with a median duration of response of 3 months. Evidence of response was apparent within one cycle of chemotherapy, and the response was maximal within two cycles. Table 1 represents the characteristics of the responding patients. There were four patients (three with recurrent local-regional disease only, and one with both local-regional disease and a lung nodule) who obtained a complete response lasting 3, 3, 16+, and 30 months. The patient who sustained a complete response for 30 months (18 months without chemotherapy) recurred in the same area of the lung (biopsy-proven) as his original disease.

The toxicity associated with this chemotherapy was generally mild. The majority of patients had vomiting associated with the administration of cisplatin, but significant vomiting was seen in only 10% of the patients and 14 patients had no vomiting. Metoclopramide was routinely administered to approximately 25% of the patients. Mild to moderate mucositis secondary to the administration of bleomycin was noted in two patients. There were also six episodes of fever associated with the administration of bleomycin. One patient had an uneventful hospitalization for leukopenia, thrombocytopenia, and fever 2 weeks after the administration of sequential MTX-5-FU. There was no evidence of renal toxicity (serum creatinine > 1.5 mg/dl) secondary to cisplatin, and there was no clinically evident bleomycin lung toxicity.

TABLE 1.—Response by entry characteristics*

	Responders	Nonresponders
ECOG performance status		
0	0	1
1	7	7
2	2	6
3	0	6
4	1	0
Distant metastases (lung)	3†	0
Previous chemotherapy	3	0
Site of disease		
Hypopharynx	3	7
Oral cavity	3	6
Nasopharynx	2	3
Oropharynx	1	4
Unknown	1	0
Median survival in mos (range)	6 (3-36)	4 (1-11)

* Unless otherwise specified, values = No. of patients.
†One complete response.

DISCUSSION

The response rate of 33% with a median duration of response of 3 months associated with alternating two effective combination chemotherapies is not superior to results reported with either combination chemotherapy utilized alone or single-agent chemotherapy in patients with recurrent SCHN (1-5). There were two long-term remissions among the four patients with complete response. Complete remissions of long duration have been reported with cisplatin alone or with various combination chemotherapies in a small percentage of patients with recurrent SCHN (6-8). The lack of increased therapeutic efficacy with this alternating combination chemotherapy in patients with advanced (post-surgery and/or radiotherapy) SCHN suggests that the currently available effective anticancer agents are not truly cross-resistant in this setting.

REFERENCES

1. WITTES RE. Combination chemotherapy of head and neck cancer in the United States. Recent Results Cancer Res 76:276-289, 1981.
2. PITMAN SW, KOWAL CD, and BERTINO JR. Methotrexate and 5-fluorouracil in sequence in squamous head and neck cancer. Semin Oncol 10 (suppl 2):15-19, 1983.
3. RINGBORG U, EWERT G, KINNMAN J, ET AL. Sequential methotrexate-5-fluorouracil treatment of squamous cell carcinoma of the head and neck. Cancer 52:971-973, 1983.
4. AMER MH, IZBICKI RM, VAITKEVICIUS VK, ET AL. Combination chemotherapy with diammine-dichloroplatinum, oncovin, and bleomycin (COB) in advanced head and neck cancer. Cancer 45:217-223, 1980.
5. DRELICHMAN A, CUMMINGS G, and AL-SARRAF M. A randomized trial of the combination of *cis*-platinum, Oncovin, and bleomycin versus methotrexate in patients with advanced squamous cell carcinoma of the head and neck. Cancer 52:399-403, 1983.

6. CARADONNA R, PALADINE W, RUCKDESCHEL JC, ET AL. Methotrexate, bleomycin, and high-dose *cis*-dichlorodiammineplatinum(II) in the treatment of advanced epidermoid carcinoma of the head and neck. Cancer Treat Rep 63:489–491, 1979.
7. AMREIN PC, FINGERT H, and WEITZMAN S. Cisplatin-vincristine-bleomycin therapy in squamous cell carcinoma of the head and neck. J Clin Oncol 1:421–427, 1983.
8. WITTES RE, CVITKOVIC E, SHAH J, ET AL. *cis*-Dichlorodiammineplatinum(II) in the treatment of epidermoid carcinoma of the head and neck. Cancer Treat Rep 61:359–366, 1977.

Phase II Trial of Gallium Nitrate in Patients With Advanced Malignant Melanoma[1,2]

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A phase II trial of gallium nitrate (250–300 mg/m²/day for 7 consecutive days) was conducted in patients with metastatic melanoma. Therapy was well-tolerated, but only one of 31 evaluable patients experienced a partial regression. Further evaluation of gallium nitrate at this dose and schedule is not warranted in patients with malignant melanoma. [Cancer Treat Rep 69:1019–1020, 1985]

Gallium 67 citrate is concentrated by a number of tumors and is clinically useful as a scanning agent for a variety of neoplasms including malignant melanoma (1). In 1971, Hart and Adamson (2) reported that gallium nitrate had considerable activity against several rodent tumors and suggested that gallium salts be explored for the treatment of human neoplasia. Further impetus for clinical evaluation of gallium nitrate as an antineoplastic agent came from the striking activity of another heavy metal compound, cisplatin.

In clinical phase I studies of gallium nitrate using intermittent high-dose schedules, dose-limiting nephrotoxicity was encountered (3,4). Similar toxicity was observed when the drug was given daily for 3 consecutive days every 2 weeks (5). In an attempt to improve the therapeutic index of this drug, a continuous iv infusion for several days was explored at Memorial Hospital. A dose of 300 mg/m²/day was tolerated by most patients, and nephrotoxicity was uncommon (6). Major objective therapeutic responses were seen in 34% of heavily pretreated patients with malignant lymphoma in that trial. Since therapeutic activity had been reported in patients with malignant melanoma in phase I trials (5,7), and since, like lymphomas, melanomas concentrate gallium 67 citrate, we conducted this phase II trial of gallium nitrate given by continuous iv infusion in patients with malignant melanoma.

PATIENTS AND METHODS

All patients had microscopic confirmation of malignant melanoma and clinical evidence of advanced disease. Criteria for eligibility included the presence of a measurable indicator lesion which was not in a previously irradiated area, performance status ≥ 50% (Karnofsky scale), wbc count ≥ 4000/mm³, platelet count ≥ 150,000/mm³, serum bilirubin < 2.5 mg/dl, and creatinine clearance ≥ 60 ml/minute/1.73 m² of body surface area. Initially, previously treated patients were entered into this study; however, the study was eventually limited to patients who had received no prior systemic chemotherapy. Before treatment, each patient had a complete physical examination with measurement of indicator lesion(s). Baseline laboratory studies included cbc, serum electrolytes, and biochemical profile; creatinine clearance; and chest radiographs. These studies were repeated prior to each course of gallium nitrate. In addition, a gallium 67 scan was required for each patient prior to administration of gallium nitrate. Other radiographic and radionuclide studies were performed when indicated by the clinical situation.

Patients with a creatinine clearance of 70 ml/minute/1.73 m² of body surface area were treated with gallium nitrate at a dose of 300 mg/m²/day for 7 days by continuous iv infusion. The dose was reduced to 250

[1]Received Dec 20, 1984; revised Mar 4, 1985; accepted Mar 8, 1985.

[2]Supported by contract N01-CM-07337 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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[4]We acknowledge the participation of Dr. S. Allen (North Shore University Hospital) and Drs. R. Winn and R. Zager (Essex County Cancer Consortium); we thank Ms. Karen Sheehan for assistance with collection of data and Ms. M. J. O'Rourke for secretarial assistance.

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mg/m²/day for patients with a lower creatinine clearance. Each patient's doses were prepared fresh daily, diluted in 1000 ml of 5% dextrose in water, and infused over 24 hours. During the 7-day infusion, serum creatinine and electrolytes were monitored at least every other day. Infusions were repeated monthly in responding patients. Unless there was unequivocal progression of disease, all patients received at least two 7-day infusions of the drug.

Standard criteria for therapeutic and toxic effects were employed.

RESULTS

Thirty-six patients were entered in this study; 30 were treated at Memorial Hospital, and six were treated in the community by cooperating CCOP (Community Clinical Oncology Program) investigators. Five patients were considered inevaluable: four died of progressive disease within 3 weeks of their first dose of gallium nitrate, and one was lost to follow-up. Of the remaining 31 patients, only four had received prior systemic cytotoxic therapy. The characteristics of the 31 evaluable patients are presented in table 1. Baseline gallium 67 scan was abnormal in all but three of the evaluable patients.

One major objective response was observed. A previously untreated 31-year-old woman with widespread lymphadenopathy and massive soft tissue involvement had almost complete resolution of metastases. This patient's initial gallium 67 scan had shown the widely disseminated disease. The partial regression lasted 7 months. Two patients had stable disease for 3+ months each. All other patients had progressive melanoma.

In general, gallium nitrate therapy was well-tolerated. Mild nausea was reported by 50% of patients and seemed to worsen toward the end of the 7-day infusion; only five patients, however, actually had vomiting. Myelosuppression was not observed; the wbc count nadir was 6900/mm³ and the platelet count nadir was 308,000/mm³. No patient experienced alopecia. In three patients with intra-abdominal metastases, fluid retention with intravascular volume depletion and transient elevation of serum creatinine occurred, but no patient suffered permanent renal injury.

Every patient had a transient reduction in serum calcium concentration. Serum calcium was measured below 8.0 mg/dl in 61% and below 7.0 mg/dl in 10% of evaluable patients. However, neither signs nor symptoms of hypocalcemia were observed.

TABLE 1.—Characteristics of 31 evaluable patients with malignant melanoma treated with gallium nitrate

Characteristic	No. of patients
Age (yrs)	
Range: 19–74	
Median: 55	
Karnofsky performance status (%)	
Range: 50–90	
Median: 80	
Sex	
Male	20
Female	11
Prior chemotherapy	
Yes	4
No	27
Dominant site of disease	
Nodes, soft tissue	16
Lung	6
Intra-abdominal, liver	9

DISCUSSION

Although one patient treated in this trial had striking regression of rapidly progressive metastatic melanoma, the overall response rate observed (3%; 95% confidence limits, 0–16%) does not justify further investigation of this dose and schedule of gallium nitrate in patients with this disease.

The objective remission rates achieved with standard agents in patients with metastatic melanoma are unsatisfactory, and toxicity remains a major problem (8). Further trials of new drugs and approaches are clearly warranted.

REFERENCES

1. MILDER MS, FRANKEL RS, BULKLEY GB, ET AL. Gallium-67 scintigraphy in malignant melanoma. Cancer 32:1350–1356; 1981.
2. HART MM, and ADAMSON RH. Antitumor activity and toxicity of salts of inorganic Group IIIa metals: aluminum, gallium, indium, and thallium. Proc Natl Acad Sci USA 68:1623–1626, 1971.
3. SAMSON MK, FRAILE RJ, BAKER LH, ET AL. Phase I–II clinical trial of gallium nitrate (NSC-15200). Cancer Clin Trials 3:131–136, 1980.
4. KRAKOFF IH, NEWMAN RA, and GOLDBERG RS. Clinical toxicologic and pharmacologic studies of gallium nitrate. Cancer 44:1722–1727, 1980.
5. BEDIKIAN AY, VALDIVIESO M, BODEY GP, ET AL. Phase I clinical studies with gallium nitrate. Cancer Treat Rep 62:1449–1453, 1978.
6. WARRELL RP, COONLEY CJ, STRAUS DJ, ET AL. Treatment of patients with advanced malignant lymphoma using gallium nitrate administered as a seven-day continuous infusion. Cancer 51:1982–1987, 1983.
7. KELSEN DP, ALCOCK N, YEH S, ET AL. Pharmacokinetics of gallium nitrate in man. Cancer 46:2009–2013, 1980.
8. LUIKART SD, KENNEALEY GT, and KIRKWOOD JM. Randomized phase III trial of vinblastine, bleomycin, and *cis*-dichlorodiammine platinum versus dacarbazine in malignant melanoma. J Clin Oncol 2:164–168, 1984.

Antitumor Activity of *trans*-Bis(salicylaldoximato)copper(II): A Novel Antiproliferative Metal Complex[1]

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***trans*-Bis(salicylaldoximato)copper(II) ($CuSAO_2$), the parent compound of a new group of antiproliferative copper(II) complexes, was studied in vivo. It had marked antitumor activity against Ehrlich ascites carcinoma in NMRI mice. Survival was considerably increased, and, in some cases, total cures were encountered. No activity against leukemia L1210 was found in female DBA/2J mice, although $CuSAO_2$ is equally effective against L1210 and Ehrlich carcinoma cells in vitro. The side effects of $CuSAO_2$ included weight loss, weakness, acrodynia, and blindness; all of these side effects were transient. [Cancer Treat Rep 69:1021–1022, 1985]**

We have recently reported (1) a novel antiproliferative agent, *trans*-bis(salicylaldoximato)copper(II) ($CuSAO_2$), which is capable of totally arresting the proliferation of Ehrlich ascites carcinoma (EAC) and leukemia L1210 cells in vitro in the concentration of about 5 μg/ml. The corresponding cobalt(II) and nickel(II) chelates had a much weaker activity. We now report the results of in vivo testing of $CuSAO_2$ against both tumor lines.

MATERIALS AND METHODS

The synthesis of $CuSAO_2$ has been described (2). Tween 20 (polyoxyethylenesorbitan monolaurate) was European Pharmacopea grade. Heparin solution (5000 IU/ml) was obtained from Medica, Helsinki, Finland.

NMRI and DBA/2J mice were obtained from the Departments of Pharmacy and Biochemistry of the University of Helsinki, respectively. They were housed at ordinary room temperature with a 12-hour light-dark cycle and were given a standard laboratory diet and tap water ad libitum.

EAC cells were maintained and propagated in female NMRI mice. After 2 weeks of propagation, the ascitic fluid was removed, heparinized (250 IU), and diluted with 0.9% aqueous NaCl. Leukemia L1210 cells were maintained in cell culture and transferred ip to female DBA/2J mice for propagation (8 days).

In all cases, $CuSAO_2$ was administered ip as a suspension in 0.9% aqueous NaCl containing 0.1% (vol/vol) of Tween 20. The injection volume was 0.2 ml/10 g of body weight.

Median survival times were calculated according to the protocols of the National Cancer Institute (3), taking Day 60 as the day of evaluation. T/C ratios (%) were calculated as stated for leukemia L1210 in the same protocols, with the exception that in EAC experiments Day 30 was taken as "Day B" and the calculation was performed as if the survivors had been discarded on Day 60.

RESULTS

$CuSAO_2$ had significant antitumor activity against EAC. The results of a multidose experiment are shown in table 1. With the best dosage (Group 9), a T/C value of 295% was obtained and the median survival time was more than twice that of the controls. Two of the eight treated mice survived over 100 days and were obviously cured, as were a few mice in other treated groups.

Weight loss and weakness after the administration of $CuSAO_2$ were considerable, and were dose-limiting. Acrodynia (in the form of swollen noses) and transient blindness of some animals were also encountered. Eventually, all side effects of the survivors totally disappeared.

In another experiment (data not shown), a very large EAC cell inoculum (about eight times the one used in the first test) was given to each NMRI mouse. When treatment was started 24 hours after tumor transplantation, significant antitumor activity (T/C 145%) was demonstrated with the daily dose 50 $mg \cdot kg^{-1}$, given for 2 days. When treatment (30, 50, or 70 $mg \cdot kg^{-1}$ daily for 2–3 days) was started 4 days after tumor transplantation, no

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[3]We thank Professor Liisa Ahtee and Associate Professor Juhani Jänne for their cooperation and valuable discussions, and Mr. Ehsan Etemadzadeh and Miss Raija Laine for skillful technical assistance.

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TABLE 1.—Activity of $CuSAO_2$ against EAC*

Group No.	Dosage in 1 day ($mg \cdot kg^{-1}$)	Treatment period (days)	No. of mice	T/C (%)	Median survival time (days)
1	Controls	—	7	—	15
2	1 × 20	3	4	127	18.5
3	1 × 20	5†	4	130	20
4	1 × 40	3†	8	151	23
5	1 × 80	1	4	103 (> 197)‡	20
6	1 × 80	2	4	(> 385)§	> 30
7	2 × 20	3	4	> 231	31.5
8	2 × 20	5†	4	135 (177)‡	13
9	2 × 40	2†	8	295	39.5
10	2 × 80	1.5†	6	Toxic	1

*Female NMRI mice weighing 23.3 ± 3.3 g were given ip about 5×10^6 EAC cells, and treatment (ip) was begun after 5 mins. The interval between 2 daily doses was 8 hrs. The controls received no treatment after injection of EAC cells.

†A longer period of treatment was planned, but because of the weakness of the mice, had to be shortened.

‡Value which is obtained if the long-term survivor is not considered as a "no-take" but as a "drug-inhibition".

§Less than 65% of mice alive on Day 5.

activity was found. In both cases the toxicity of $CuSAO_2$ was considerably reduced in comparison to the first EAC experiment, and no weight loss was observed.

No activity against leukemia L1210 was found when 30, 40, or 50 $mg \cdot kg^{-1}$ of $CuSAO_2$ was administered ip once a day on Days 1-3 to female DBA/2J mice that had on Day 0 received ip 1×10^6 L1210 cells each. The mean survival times in days were 17.1 ± 1.6 for the untreated controls, 14.0 ± 5.7 for the 30-$mg \cdot kg^{-1}$ group, 14.2 ± 2.2 for the 40-$mg \cdot kg^{-1}$ group, and 14.8 ± 2.2 for the 50-$mg \cdot kg^{-1}$ group.

DISCUSSION

$CuSAO_2$ is equally active against EAC and leukemia L1210 in vitro (1). Its lack of activity against the latter in vivo suggests that this compound, which is almost insoluble in water, possibly is not spread well enough systemically. Therefore, we are now synthesizing and testing more soluble derivatives of the compound. The 4-hydroxyl derivative of $CuSAO_2$, which is considerably more hydrophilic than the parent compound, has already been shown to be active in vitro (1). Studies on the toxicity and mechanism of action of $CuSAO_2$ are also under way (4).

REFERENCES

1. LUMME P, ELO H, and JANNE J. Antitumor activity and metal complexes of the first transition series. *trans*-Bis(salicylaldoximato)copper(II) and related copper(II) complexes, a novel group of potential antitumor agents. Inorganica Chimica Acta, Bioinorganic Chemistry Articles and Letters 92:241-251, 1984.
2. LUMME P, and KORVOLA M-L. Studies on coordination compounds VI. Thermogravimetric, differential thermogravimetric, differential thermal analysis and mass spectrometric studies of some cobalt(II), nickel(II) and copper(II) salicylaldoximates, 2-indolecarboxylates and 2-thiophenecarboxylates. Thermochim Acta 13:419-439, 1975.
3. GERAN RI, GREENBERG NH, MACDONALD MM, ET AL. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemother Rep Part 3, vol 3(2):1-103, 1972.
4. LUMME PO, and ELO HO. Antitumor activity of metal complexes and enzymatic homogeneous catalysis of vital processes of tumor cells. *In* Proceedings of the 4th International Symposium on Homogeneous Catalysis, Leningrad, 1984, Book II, p 42.

Clinical Evaluation of High-Dose Cytarabine and Cisplatin in Recurrent Cervical Carcinoma[1]

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The response of uterine cervical carcinoma to chemotherapy is usually reported in the range of 10%–30% (1). Higher response rates are possible with cisplatin (2) or combination therapy (3).

Cytarabine (ARA-C) has little antitumor effect against solid tumors but a marked synergistic effect was observed in vitro in combination with cisplatin (4). We therefore initiated a phase II study with cisplatin and ARA-C as induction therapy in patients with recurrent cervical carcinoma who were not candidates for surgery or further irradiation.

PATIENTS AND METHODS

Patients with recurrent cervical carcinoma were considered eligible if there was no significant renal or hepatic impairment, no prior chemotherapy, and adequate bone marrow function. The phase II study was started in April 1981 after the maximum tolerated dose of the combination had been determined from an escalating dose schedule. ARA-C was administered at 1200 mg/m^2 (1800 mg maximum dose) in 500 ml of 5% dextrose in water, and cisplatin was administered at 110 mg/m^2 (165 mg maximum dose) in 1 L of 0.45% NaCl plus 50 g of mannitol. Both agents were administered iv in opposite arms over 3–4 hours, and treatment was repeated every 3–4 weeks provided the wbc count was $\geq$ 4000/mm^3, platelet count was $\geq$ 150,000/mm^3, and serum creatinine was $\leq$ 1.5 mg/dl. Dosages were reduced by 25%–50% for blood cell counts less than the above, and all chemotherapy was discontinued for granulocyte counts $\leq$ 500/mm^3 or serum creatinine $\geq$ 3 mg/dl and bilirubin $\geq$ 3 mg/dl. World Health Organization criteria for response were used.

Four induction courses of ARA-C and cisplatin were planned. Continuation of ARA-C and cisplatin was permitted in the presence of complete response and in the absence of nephrotoxicity or neurotoxicity. Treatment was continued with cyclophosphamide at 1500 mg iv at 4-week intervals.

RESULTS

Thirty-two patients were entered and treated. The median age was 53 years (range, 24–90). Twenty-three of these patients received at least two courses of treatment. All patients had received prior pelvic irradiation, but none had received chemotherapy. Twenty patients had recurrent or persistent disease in the pelvis and 18 had distant metastases with or without regional disease. A total of 93 courses of ARA-C and cisplatin were administered and 43 courses of cyclophosphamide were administered. The median number of courses of ARA-C and cisplatin was 3.5 (range, one to six courses), while the median number of courses of cyclophosphamide was 2.0 (range, one to ten courses). Only 11 patients received cyclophosphamide.

Of 32 patients, seven (22%) achieved response: two complete and five partial. There were eight patients with stable disease. A 30% response rate was observed in patients who received at least two courses of treatment. Relevant characteristics of responding patients are shown in table 1. The first patient in table 1 is the only surviving patient under this protocol. The second patient received two courses of ARA-C and cisplatin and had a partial response; however, at her request, no further chemotherapy was administered, and she died 25 months after start of treatment. The remaining five responding patients survived from 10 to 27 months. The median survival for the group was 7.2 months: for responders, 19.5 months; for patients with stable disease, 10.2 months; and for patients with progressive disease, 4.1 months. All of the responses occurred while patients were receiving ARA-C and cisplatin, and no additional responses occurred when the treatment was changed to cyclophosphamide or other chemotherapy. The median

[1]Received May 3, 1984; revised Feb 15, 1985; accepted Mar 8, 1985.

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duration of response was 9 months. Survival appeared to be unrelated to the interval of time between the original diagnosis and the recurrence or to stage, grade, recurrence site, or previous radiation field.

Toxicity

One patient died 24 hours following chemotherapy and tumor emboli were found at autopsy. Death was probably unrelated to treatment.

Six patients required hospitalization for significant nausea and vomiting; temperature elevations > 39°C occurred in 17 of 93 courses and those > 40°C occurred in three courses. Six patients had a > 50% decrease in creatinine clearance and two additional patients had a > 50% increase in serum creatinine. It is possible that progressive urinary tract obstruction may have contributed to the progressive renal failure in some patients. Wbc counts decreased to between 3000 and 4000/mm^3 at the time of the next treatment dose in seven patients but platelet counts remained > 100,000/mm^3. Hemoglobin values fell to < 9 g in seven patients.

DISCUSSION

The previous in vitro demonstration of a marked increment in cisplatin's lethal effect on tumor cells when ARA-C (4) or natural nucleosides (5) were administered simultaneously provided the rationale for combining these agents in clinical trials. A potential advantage of such a combination may be an enhanced therapeutic index for the use of chemotherapeutic agents without significant overlapping toxicity.

Two previous clinical studies used a combination of ARA-C and cisplatin for esophageal and metastatic colorectal cancer, respectively (6,7). In both instances, cisplatin was used at a much lower bolus dose (20 mg/m^2) than in the present study, while ARA-C was administered over a longer period of time. In vitro studies had shown that by administering the maximal dose of cytarabine at once, DNA cross-links could be increased by the cisplatin without exerting an inhibition of DNA synthesis induced by the long-term administration of cytarabine. Thus, the previously cited studies predictably reported a high rate of myelotoxicity with negligible antitumor effect.

In a previous unpublished phase I study, the highest tolerated dose of ARA-C and cisplatin was 1200 and 110 mg/m^2, respectively. Apart from the expected gastrointestinal toxicity and nephrotoxicity due to the cisplatin, several cases of severe but transient hyperpyrexia occurred. Myelosuppression was acceptable and these dosages were therefore used in the phase II study. No apparent advantage resulted from this combination when compared to other agents used alone or in combination in this disease. Our in vitro studies showed a synergistic increase in cytotoxicity which was linearly dose-dependent for each agent. Enhanced in vitro cytotoxicity from ARA-C required a concentration > 2 mM which could not have been achieved with our treatment dose administered iv over 3 hours. It is unlikely that the level of desired synergism can be realistically achieved by a further increase in dose by this route, although the direct delivery of drugs via the arterial route in selected patients with recurrent pelvic disease might provide an opportunity to test the concepts established in vitro.

TABLE 1.—Characteristics of patients with response or stable disease

Patient No.	Tumor site*	Courses of ARA-C and cisplatin	Courses of cyclophosphamide	Response†	Duration (mos)	Survival (mos)
1	L	4	8	CR	36	38‡
2	P, L	2	0	PR	24	25§
3	P	6	10	CR	12	20
4	P, Abd	5	0	NC	4	12
5	L	5	2	NC	6	10
6	P	6	3	PR	9	14
7	P	4	2	NC	6	11
8	P	4	1	PR	3	14
9	L	5	2	NC	8	11
10	L	3	0	NC	2	3
11	P, L	4	10	NC	14	18
12	P	5	0	PR	8	27
13	P	3	0	NC	2	7
14	L, Scn	5	2	PR	5	10
15¶	P, Abd	1	0	NC	7	11

*L = lung, P = pelvis, Abd = abdomen, and Scn = supraclavicular node.
†CR = complete response; PR = partial response; and NC = no change (stable).
‡Alive.
§Rapid progression at outside hospital.
¶Inadequate trial.

REFERENCES

1. WASSERMAN TH, and CARTER SK. The integration of chemotherapy into combined modality treatment of solid tumors. Cancer Treat Rev 4:25–46, 1977.
2. PRESTAYKO AW, D'AOUST JC, ISSELL BF, ET AL. Cisplatin (*cis*-diamminedichloroplatinum II). Cancer Treat Rev 6:17–39, 1979.
3. ALBERTS DS, MARTIMBEAN PW, SURWIT EA, ET AL. Mitomycin-C, bleomycin, vincristine, and *cis*-platinum in the treatment of advanced, recurrent squamous cell carcinoma of the cervix. Cancer Clin Trials 4:313–316, 1981.
4. BERGERAT JP, DREWINKO B, CORRY P, ET AL. Synergistic lethal effect of *cis*-dichlorodiammineplatinum and 1-beta-D-arabinofuranosylcytosine. Cancer Res 41:25–30, 1981.
5. DREWINKO B, CORRY P, BERGERAT JP, ET AL. The lethal activity of platinum compounds in combination with pyrimidine derivatives. *In* Proceedings of Cisplatin: Current Status and New Developments. Third Annual Bristol Symposium. New York, Academic Press, 1980, pp 37–55.
6. EISENBERGER M. PEREIRAS R, SOLOMON J, ET AL. Combination chemotherapy with *cis*-dichlorodiammineplatinum (DDP) and cystosine arabinoside (CA) given by intra-arterial (IA) or intravenous (IV) infusion in patients (PTS) with metastatic colorectal cancer. Proc Am Assoc Cancer Res and ASCO 22:453, 1981.
7. HILL JB, BADER J, MYERS C, ET AL. Pilot study of low dose continuous infusion of ARA-C and pulsed low dose cisplatinum in esophageal carcinoma. Proc ASCO 1:111, 1982.

Therapy for Chronic Myelogenous Leukemia in Blast Crisis With Etoposide and 5-Azacitidine Administered by Continuous Infusion: A Cancer and Leukemia Group B Study[1]

Charles A. Schiffer,[2,*] Keaven Anderson,[3] Morton Coleman,[4] and Janet Cuttner[5]

A variety of agents with activity in acute nonlymphocytic leukemia have been ineffective in prolonging survival in patients with either the accelerated or blast crisis phases of chronic myelogenous leukemia. A recently published study suggested that 5-azacitidine and etoposide (VP16-213) administered in combination may have produced improved results, with a median overall survival of 161 days and an overall response rate of 58% (16 responses among 27 patients). Only one patient had a complete response, and the duration of the 15 partial responses was a few months. Five patients in this study, including the complete responder, did not have a chronic phase and presented with "Philadelphia-positive acute leukemia." The present study was done using higher doses of both drugs because aplasia was not achieved in most patients in the earlier study. In addition, the 5-azacitidine was administered by continuous infusion in an attempt to ameliorate the gastrointestinal side effects (1).

PATIENTS AND METHODS

Thirty-five patients with chronic myelogenous leukemia in blast crisis were entered in the study (2,3). Patient characteristics are summarized in table 1. Approximately one-third of the patients had received prior therapy for blast crisis, which in most cases consisted of vincristine and prednisone-based regimens. The overall performance status of the group was very poor, with 23 of the patients having Cancer and Leukemia Group B (CALGB) grades 3 or 4. Induction treatment consisted of 5-azacitidine (250 mg/m^2/day for 5 days by continuous iv infusion) plus VP16-213 (100 mg/m^2/day for 5 days by iv infusion over 1 hour). The 5-azacitidine was suspended in 500–1000 ml of Ringer's lactate, with new bottles prepared every 8 hours. If aplasia was not achieved, repeat courses of the therapy were administered on Days 14 or 21.

RESULTS

Two patients who received < 12 hours of total therapy were considered nonevaluable for response. Both patients had hypotensive episodes during the first day of the 5-azacitidine infusion. Although the etiologies could not be ascertained in these patients, in one patient the hypotension may have been related to iv thorazine given as premedication. A total of six patients, including one of those in whom hypotension developed, died within 1 week of starting therapy. Three of these patients had very poor performance status when treatment was initiated and the causes of death were combinations of infection and hemorrhage. One complete response (2,3) was noted among the remaining 28 patients. This was a 52-year-old male who had never had an identifiable chronic phase but who presented with "Philadelphia-positive," terminal transferase negative acute leukemia. The patient received one course of maintenance therapy at 50% of the induction doses and relapsed 60 days after achieving an M-1 marrow. One other patient achieved an M-1 marrow and normal blood cell count but relapsed with blasts in the peripheral blood within the following 2 weeks. Although cytoreduction was achieved in most patients, no other partial or complete responses were seen. The median overall survival was 1.3 months.

As expected, myelosuppression was severe, with essentially all patients developing severe leukopenia and thrombocytopenia. Gastrointestinal side effects were quite tolerable, with only 3% of the patients developing either CALGB grade 3 or 4 nausea and vomiting. Renal tubular acidosis, defined as a fall in serum bicarbonate to < 20 mEq/L in association with an alkaline urine, devel-

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	35
Sex	
Male	17
Female	18
Median age in yrs (range): 51 (23–83)	
Performance status (CALGB)	
0–1	6
2	6
3–4	23
Prior treatment for blast crisis	12
Terminal transferase positive	9/19*
Median wbc count: 45,000/μl	
Median platelet count: 70,000/μl	
Fever > 100°C: 66%	
Early death (< 7 days)	6
Nonevaluable	2

* No. of patients with positive test/No. tested.

oped in five patients. This is probably an underestimate of the frequency of this complication which has been described following 5-azacitidine therapy (4) because complete data related to this complication were not available on most patients. This was not accompanied by renal insufficiency and in general reversed within 1 week of onset. Nonetheless, in some patients who developed other complications such as significant infections with hypotension, the pre-existing acidosis may have complicated the further management to some degree. Transient pericardial rubs with chest pain were noted in two patients after completion of the infusions. These complications resolved spontaneously; no definite etiology was determined and it was unclear whether these were drug-related. Hepatic necrosis and areas of marrow necrosis were found at autopsy in two separate patients. Both patients had severe infections and were receiving multiple medications, and it is doubtful that these findings were caused by the chemotherapeutic drugs themselves.

CONCLUSION

The overall results of this study are quite disappointing and unfortunately in keeping with previous results obtained in the treatment of chronic myelogenous leukemia in blast crisis (3,5,6). It is likely that, in part, the results from the present study were inferior to the previously published results with 5-azacitidine and VP16-213 because of the poor overall performance status and the prior treatment status of the patients entered. Nonetheless, because of the low response rate and the absence of any long-term responders, this regimen cannot be recommended for the treatment of chronic myelogenous leukemia in blast crisis.

REFERENCES

1. ISRAILI H, VOGLER WR, MINGIOLI ES, ET AL. Disposition and pharmacokinetics in humans of 5-azacitidine administered intravenously as a bolus or by continuous infusion. Cancer Res 46:1453–1461, 1976.
2. SCHIFFER CA, DEBELLIS R, KASDORF H, ET AL. Treatment of the blast crisis of chronic myelogenous leukemia with 5-azacitidine and VP-16-213. Cancer Treat Rep 66:267–271, 1982.
3. COLEMAN M, SILVER RT, PAJAK TF, ET AL. Combination chemotherapy for terminal phase chronic granulocytic leukemia: Cancer and Acute Leukemia Group B studies. Blood 55:29–36, 1980.
4. PETERSON BA, COLLINS AJ, VOGELZANG NJ, ET AL. 5-Azacytidine and renal tubular dysfunction. Blood 57:182–185, 1981.
5. SPIERS ASD, GOLDMAN JM, CATOVSKY D, ET AL. Multiple-drug chemotherapy for acute leukemia: the trampcol regimen: results in 86 patients. Cancer 40:20–29, 1977.
6. VALLEJOS CS, TRUJILLO JM, CORK A, ET AL. Blastic crisis in chronic granulocytic leukemia: experience in 39 patients. Cancer 34:1806–1812, 1974.

Phase II Evaluation of Aclarubicin in Advanced Breast Cancer: A Southeastern Cancer Study Group Trial[1]

Jon P. Gockerman,[2,*] Marilyn Raney,[3] and Theresa Logan[3]

A number of anthracycline derivatives are being evaluated with the hope of finding one with a better therapeutic index than doxorubicin. Aclarubicin (aclacinomycin A) appeared to be such an agent in animal tumor models and phase I studies (1-3). This study was undertaken to evaluate the therapeutic efficacy of aclarubicin in metastatic breast cancer using two different dosage schedules: a weekly dose, based on animal data suggesting an improved cell kill, and an every-3-week schedule similar to the schedule used for doxorubicin. Only patients without previous anthracycline therapy were treated, to eliminate the question of cross-resistance as a cause of treatment failure.

MATERIALS AND METHODS

Patient selection for both schedules was identical, and required the following: biopsy-proven metastatic adenocarcinoma of the breast, no previous anthracycline therapy, Karnofsky performance status ≥ 50%, and the presence of at least one measurable lesion. Individual institutions were randomly assigned a dosage schedule to be used for all patients entered from that institution. Twenty-four patients were entered into a weekly schedule of aclarubicin (70 mg/m^2 by iv push) for 6 weeks, and were then evaluated. If a response or stabilization of disease was achieved, therapy was continued until disease progression. Nine other patients were entered into a schedule of aclarubicin (100 mg/m^2 by iv push) given every 3 weeks. These patients were re-evaluated 3 weeks after their second dose. Eighteen of the 24 patients on the weekly schedule were evaluable for toxicity and 17 were evaluable for response. The inevaluable cases were due to: refusal of drug (two patients); protocol errors by investigator (three); and inadequate documentation (one). Eight of the nine patients on the every-3-week schedule were evaluable for toxicity and seven were evaluable for response. The inevaluable cases were due to patients' refusal to continue therapy. The required cardiac evaluation was an ECG prior to and every 12 weeks during treatment and a physical examination at the time of each chemotherapy dose. Response was assessed by published guidelines (4).

RESULTS

Toxic effects.—The most serious toxic effect was hematologic, with severe neutropenia seen in three patients in both the weekly (17%) and the every-3-week schedule (38%) (table 1). The most frequent toxic effect was nausea and vomiting. The weekly schedule had 13 patients (72%) with moderate to severe nausea and vomiting; the every-3-week schedule had five patients (63%) with moderate nausea and vomiting. Only one patient in each group had mild to moderate hair loss. There was no reported cardiotoxicity and there were no reported drug extravasations.

Response.—There were no responses (complete or partial) seen in the 17 patients evaluable on the weekly aclarubicin schedule. Four patients had stable disease at the time of evaluation; all continued on therapy and showed disease progression within 2-6 months. Of the seven patients evaluable for response on the every-3-week schedule, six showed disease progression at the time of initial evaluation and one had stable disease. This patient's disease progressed in 5 months on continued therapy.

DISCUSSION

Aclarubicin showed no evidence in this trial of cardiotoxicity and was associated with minimal hair loss. The drug produced moderate to severe bone marrow and gastrointestinal toxicity, in the face of a failure to produce evidence of disease regression in metastatic breast cancer in patients who had not been previously treated with an anthracycline. Aclarubicin is not an effective therapy in metastatic breast cancer when given on weekly schedules using the dose in this protocol. The patient population is too small on the every-3-week

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TABLE 1.—Patient characteristics and toxic effects*

	Treatment schedule	
	Weekly	Every 3 wks
Total	24	9
Median age in yrs (range)	57.9 (37.9–79.4)	56.5 (33.7–71.0)
Median Karnofsky performance status (range)	70 (60–90)	75 (50–100)
Prior hormone therapy (total)	13	4
Estrogen/antiestrogen	12	3
Androgen	4	1
Progesterone	4	1
Glucocorticoid	1	0
No data	0	2
Prior chemotherapy (total)	24	7
CMF†	22	6
CMFVP‡	2	1
Single agent (other than doxorubicin)	6	2
No data	0	2
Dominant site of disease		
Soft tissue	18	8
Bone	13	4
Visceral	12	6
Estrogen receptor status		
Negative	8	2
Positive	5	2
Unknown	11	5
Granulocyte nadir within treatment interval (cells/mm^3)		
1500–1899	2	2
1000–1499	2	1
500–999	5	0
< 500	3	3
Nausea and vomiting		
Controllable (moderate)	8	5
Intractable (severe)	5	0

* Unless otherwise specified, values = No. of patients.

†CMF = cyclophosphamide, methotrexate, and 5-FU.

‡CMFVP = cyclophosphamide, methotrexate, 5-FU, vincristine, and prednisone.

schedule to make a final conclusion.

REFERENCES

1. Clinical Brochure. Aclacinomycin A (NSC-208734). Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, July, 1979.
2. VAN ECHO DA, WHITACRE MY, AISNER J, ET AL. Phase I trial of aclacinomycin A. Cancer Treat Rep 66:1127–1132, 1982.
3. OGAWA M, INAGAKI J, HORIKOSHI N, ET AL. Clinical study of acalacinomycin A. Cancer Treat Rep 63:931–934, 1979.
4. HAYWARD JL, CARBONE PP, HEUSON JC, ET AL. Assessment of response to therapy in advanced breast cancer. Cancer 39:1289–1294, 1977.

Phase II Study of Recombinant Alpha Interferon in the Treatment of Advanced Non-Small Cell Lung Carcinoma[1,2]

Steven M. Grunberg,[3,*] Raymond A. Kempf,[3] Loretta M. Itri,[4] Cheryl L. Venturi,[3] William D. Boswell, Jr,[3] and Malcolm S. Mitchell[3]

Over the last 10 years, numerous clinical studies have been performed with extracted natural alpha interferon, a relatively crude material of only 1% purity available in limited amounts (1). Using this material at 3 million units/day, both Krown et al (2) and Figlin and Sarna (3) reported no activity in non-small cell lung cancer. However, genetic engineering techniques have now made available large quantities of pure recombinant alpha interferon (4). We therefore performed a phase II trial using recombinant alpha interferon at maximum tolerated doses in advanced non-small cell lung carcinoma.

PATIENTS AND METHODS

Eligibility criteria included advanced measurable non-small cell lung carcinoma; Karnofsky performance status ⩾ 50%; and adequate hematologic, renal, and hepatic functions. Patients with severe heart disease, patients who had received prior chemotherapy or interferon therapy, and patients receiving simultaneous therapy with nonsteroidal anti-inflammatory agents or corticosteroids were ineligible. Recombinant alpha interferon, obtained from Hoffmann-La Roche Inc, was administered at 50 million units/m^2 im three times a week. Serial doses were reduced to 50% and 10% of the starting dose in the event of toxicity.

Response was evaluated according to World Health Organization criteria (5) every 4 weeks. Patients removed from therapy for reasons other than progressive disease prior to Day 29 of therapy were not considered evaluable for response. All patients were evaluated for toxicity at every clinic visit.

RESULTS

Twelve of 16 patients who were entered in the study were evaluable for response (table 1). Of the four ineval-

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	16
Median age in yrs (range): 63 (35-77)	
Karnofsky performance status (%)	
90-100	5
70-80	9
50-60	2
Evaluable for response	12
Histologic subtype	
Adenocarcinoma	6
Squamous carcinoma	3
Large cell carcinoma	1
Adenosquamous carcinoma	1
Poorly differentiated carcinoma	1
Response	
Complete	0
Partial	0
Minimal	1
Stable disease	6
Progressive disease	5
Evaluable for toxicity	
Acute	16
Chronic	12
Toxicity (acute)	
Fever	16
Chills	16
Myalgias	16
Cardiac	4
Myocardial infarction	1
Ventricular tachycardia	1
Ventricular ectopic beats	2
Toxicity (chronic)	
Hypotension	10
Fatigue (severe)	10
Elevated liver function tests	6
Confusion	3
Paresthesias (mild)	3

[1]Received Oct 11, 1984; revised Mar 4, 1985; accepted Mar 15, 1985.
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uable patients, one had a myocardial infarction, another discontinued therapy after two doses due to severe fatigue, a third developed increasing ventricular arrhythmias, and the fourth developed anaerobic cellulitis at a biopsy site. No complete or partial responses were seen. One patient achieved minimal response, with a 50% tumor shrinkage lasting < 4 weeks. Six patients achieved stable disease of short duration (median, 6 weeks). Five had progressive disease.

All patients had acute toxic effects including fever, chills, and occasional myalgias during the 24-hour periods after the first injections. These toxic effects diminished during the first week of therapy. The dose-limiting chronic toxic effect was severe fatigue requiring dose reduction in ten of the 12 fully evaluable patients. Duration of therapy at 100% dose ranged from 1 day to 4 weeks (median, 2). Transient elevation of liver function tests, transient confusion, and mild paresthesias were also noted.

Cardiovascular toxicity was commonly seen. One patient with a history of angina had a silent myocardial infarction on Day 1. A second patient had increasing ventricular arrhythmias after 1 week of therapy. Two patients had rare ventricular ectopy during the first days of therapy. In addition, the gradual development of hypotension not relieved by volume repletion was noted in ten patients.

DISCUSSION

We have performed a phase II study of the treatment of non-small cell lung carcinoma using maximum tolerated doses of recombinant alpha interferon. No complete or partial responses were achieved among 12 evaluable patients despite significant toxicity. We conclude that recombinant alpha interferon at this dose and schedule does not have significant single-agent activity in advanced non-small cell lung carcinoma.

REFERENCES

1. KIRKWOOD JM, and ERNSTOFF MS. Interferons in the treatment of human cancer. J Clin Oncol 2:336–352, 1984.
2. KROWN SE, STOOPLER MB, CUNNINGHAM-RUNDLES S, ET AL. Phase II trial of human leukocyte interferon in non-small cell lung cancer. Proc Am Assoc Cancer Res 21:179, 1980.
3. FIGLIN RA, and SARNA GP. Human leukocyte interferon (alpha IFN): phase II trials in non-small cell lung cancer (NSCLC) and adenocarcinoma of the colon/rectum. Proc ASCO 2:45, 1983.
4. GOEDDEL DV, YELVERTON E, ULLRICH A, ET AL. Human leukocyte interferon produced by *E. coli* is biologically active. Nature 287:411–416, 1980.
5. MILLER AB, HOOGSTRATEN B, STAQUET M, ET AL. Reporting results of cancer treatment. Cancer 47:207–214, 1981.

Phase II Study of Mitoxantrone, Aclarubicin, and Diaziquone in the Treatment of Non-Small Cell Lung Carcinoma: An Eastern Cooperative Oncology Group Study[1,2]

David S. Ettinger,* Dianne M. Finkelstein, Gregory R. Harper, John C. Ruckdeschel, Alex Yuang-Chi Chang, Fernando J. Camacho, John C. Marsh, Robert Silber, and Janet M. Wolter[2]

This randomized phase II study was performed to evaluate the therapeutic efficacy of mitoxantrone (DHAD), aclarubicin (ACM-A), and diaziquone (AZQ) in patients with histologically confirmed advanced non-small cell lung cancer (1-3).

MATERIALS AND METHODS

One hundred thirty patients were entered in the study between March 1982 and July 1983. One hundred ten patients were evaluable for response and/or toxicity. There were 12 (9%) cancellations prior to starting therapy. This represents four patients who died prior to treatment, three who refused treatment, and five who were found ineligible before treatment began. There were eight (6%) patients who were found to be ineligible after treatment had begun (prior carmustine too recent, one patient; inadequate blood cell counts, two; received radiotherapy to lesion being followed on study, one; lung cancer not proven, three; and disease not evaluable, one).

Patients were eligible for this study if they had measurable or evaluable disease; an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2; adequate bone marrow, liver, and renal functions; no radiotherapy to measurable or evaluable disease; and no chemotherapy for at least 3 weeks prior to entry in the study (6 weeks for prior nitrosourea chemotherapy). Eligible patients were stratified by histologic type, ECOG performance status, and prior chemotherapy. Treatments were assigned by telephone at a central randomization office; either DHAD (12 mg/m^2), ACM-A (100 mg/m^2), or AZQ (40 mg/m^2) was infused iv over 30 minutes every 3 weeks. Each drug was given until there was clinical evidence of disease progression.

Standard ECOG criteria for solid tumor response and toxicity were used (4).

RESULTS

Table 1 shows characteristics of the 110 evaluable patients by treatment. No complete or partial responses were observed in the patients who received DHAD; one partial response was observed in a patient who received ACM-A who had had no prior chemotherapy, and one partial response was observed in a patient who received AZQ who had had prior chemotherapy.

The survival ranged from 7 to 568 days (AZQ), with a median of 13.0 weeks (DHAD, 12.4; ACM-A, 11.8; and AZQ, 16.9). Five patients have survived > 1 year (three treated with AZQ; and one treated with DHAD).

Treatment complications were reported according to ECOG standard criteria by degree (none, mild, moderate, severe, life-threatening, and lethal). There were 19 life-threatening complications (18 patients treated with all three drugs had hematologic toxicity; and one treated with AZQ had anaphylaxis) and two lethal complications

[1]Received Oct 17, 1984; revised Jan 11, 1985; accepted Mar 8, 1985.

[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in this study: The Johns Hopkins Oncology Center, Baltimore, MD (*D. S. Ettinger*, CA-16116); Dana-Farber Cancer Institute, Boston, MA (*D. M. Finkelstein*, CA-23318); Albany Medical College, NY (*G. R. Harper* and *J. C. Ruckdeschel*, CA-06594); University of Rochester Cancer Center, NY (*A. Y.-C. Chang*, CA-11083); Albert Einstein College of Medicine, Bronx, NY (*F. J. Camacho*, CA-14958); Yale School of Medicine, New Haven, CT (*J. C. Marsh*, CA-15485); New York University Medical Center, New York, NY (*R. Silber*, CA-16395); and Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (*J. M. Wolter*, CA-10948). Other participating institutions include: American Oncologic Hospital, Philadelphia, PA (CA-18281); Brown University, Providence, RI (CA-15947); Case Western Reserve University, Cleveland, OH (CA-14548); Chicago Medical School, IL (CA-14144); Hahnemann Medical College, Philadelphia, PA (CA-13611); Mayo Clinic, Rochester, MN (CA-13650); Medical College of Ohio, Toledo; University of Minnesota, Minneapolis (CA-20365); Northwestern University Medical Center, Chicago, IL (CA-17145); University of Ottawa, Ontario, Canada; Pennsylvania Hospital, Philadelphia (CA-13613); Hospital of the University of Pennsylvania, Philadelphia (CA-15488); University of Pittsburgh, PA (CA-18653); Roswell Park Memorial Institute, Buffalo, NY (CA-12296); University of Pretoria, Republic of South Africa (CA-21692); Tufts University, Walpole, MA (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, OK; and Wisconsin Clinical Cancer Center, Madison (CA-21076).

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TABLE 1.—Patient and disease characteristics by treatment

Characteristic	No. of patients (%) DHAD	ACM-A	AZQ
Entered	49	40	41
Evaluable	39	35	36
Age (yrs)			
< 50	3	6	5
50–69	34	27	26
> 70	2	2	5
Sex			
Male	31	26	24
Female	8	9	12
Extent of disease			
Limited	5	0	2
Extensive	34	35	34
Initial performance status			
0, 1	24	27	25
2	15	8	11
Histology			
Epidermoid carcinoma	18	11	16
Adenocarcinoma	12	15	16
Large cell carcinoma	8	7	4
Other	1	1	0
Unknown	0	1	0
Prior weight loss			
None	17	14	13
< 10%	15	15	18
> 10%	6	4	5
Unknown	1	2	0
Prior chemotherapy			
No	11	6	8
Yes	28	29	28
Prior radiotherapy			
No	20	20	21
Yes	19	15	15
Prior surgery			
No	34	30	28
Yes	5	5	8
Response			
Complete	0	0	0
Partial	0	1	1
Stable disease	6	3	2
Disease progression	27	28	30
Unknown	2	0	0
Unevaluable*	4	3	3
CNS progression by histology			
Epidermoid carcinoma	0	2	4
Adenocarcinoma	0	0	1
Large cell carcinoma	2	1	0
Other	0	0	0
Treatment complications			
Unevaluable†	1 (3)	1 (3)	0
None	3 (8)	2 (6)	2 (6)
Mild	7 (18)	11 (31)	1 (3)
Moderate	11 (29)	7 (20)	7 (19)
Severe	15 (39)	11 (31)	10 (28)
Life-threatening	1 (3)	3 (9)	15 (42)
Lethal	1 (3)	0	1 (3)

* Lost to follow-up or early death (before 21 days).

†One patient treated with DHAD had no follow-up data; 1 treated with ACM-A had dosage escalation.

(DHAD, hematologic toxicity; and AZQ, infection). Forty-two percent of the patients receiving AZQ experienced life-threatening hematologic toxicity (five had thrombocytopenia; one had leukopenia; and eight had both).

DISCUSSION

In this phase II ECOG study of DHAD, ACM-A, and AZQ in the treatment of non-small cell lung cancer, two partial responses (one patient treated with ACM-A; and one treated with AZQ) were observed in the 110 patients. The data from this study suggest that these three drugs are inactive against non-small cell lung cancer. However, since most of the patients treated had had prior chemotherapy, the potential efficacy of these drugs in previously untreated patients was not completely addressed.

REFERENCES

1. ALBERTS DS, GRIFFITH KS, GOODMAN GE, ET AL. Phase I clinical trial of mitoxantrone: a new anthracenedione anticancer drug. Cancer Chemother Pharmacol 5:11–15, 1980.
2. OKI T, MATSUZAWA Y, YOSHIMOTO A, ET AL. New antitumor antibiotics aclacinomycin-A and B. J Antibiot 28:830–834, 1975.
3. GRIFFIN JP, NEWMAN RA, MCCORMACK JJ, ET AL. Clinical and clinical pharmacologic studies of aziridinylbenzoquinone. Cancer Treat Rep 66:1321–1325, 1982.
4. OKEN MM, CREECH RH, TORMEY DC, ET AL. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649–655, 1982.

Phase II Study of Diaziquone in Advanced Soft Tissue and Bony Sarcoma: A Southwest Oncology Group Study[1,2]

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Diaziquone (AZQ) is a rationally synthesized bifunctional quinone which has shown substantial activity in several animal tumor models (1,2). In human phase I testing, the dose-limiting toxic effect of AZQ was found to be myelosuppression; mild gastrointestinal toxicity has also been seen (3,4). In the present study, the Southwest Oncology Group (SWOG) evaluated the activity of AZQ in advanced soft tissue and bony sarcomas.

MATERIALS AND METHODS

Patients with histologically confirmed advanced soft tissue and bony sarcomas were treated between August 25, 1982, and May 2, 1983. Eligibility criteria included the following: a condition not amenable to surgery or treatment with SWOG studies of higher priority; a life expectancy of at least 6 weeks; ambulatory performance status (SWOG grade ≤ 2); pretreatment wbc count $\geq 4000/\mu l$; platelet count $\geq 100{,}000/\mu l$; serum creatinine ≤ 2.0 mg/dl; no chemotherapy or radiation therapy within 3 weeks (6 weeks in the case of nitrosourea or mitomycin); measurable disease; and informed consent.

Good-risk patients (defined as no prior nitrosourea or mitomycin therapy, good tolerance to prior chemotherapy, and no prior extensive pelvic or mediastinal irradiation) received an initial AZQ dose of 40 mg/m^2 as a slow iv injection through the side tubing of a running iv line every 3 weeks. All other patients were considered poor-risk and received an initial dose of 30 mg/m^2. The dose of AZQ could be escalated to a maximum of 45 mg/m^2. Standard response criteria defined in previous trials were used (5).

TABLE 1.—Patient characteristics and treatment results*

	Soft tissue sarcomas	Bony sarcomas	Total
Entered	45	6	51
Fully evaluable	39	6	45
Males:females	21:18	3:3	24:21
Histologic diagnoses			
Leiomyosarcoma	20	—	20
Malignant fibrous histiocytoma	4	—	4
Liposarcoma	5	—	5
Fibrosarcoma	5	—	5
Other	5	—	5
Osteogenic sarcoma	—	4	4
Chondrosarcoma	—	2	2
Total	39	6	45
Previous treatment			
Chemotherapy only	24	2	26
Chemotherapy & irradiation	15	4	19
Radiation only	0	0	0
	0	0	0
Total	39	6	45
No. of previous chemotherapy programs (mean)	1.4	2.2	1.5
Response			
Partial	1 (4 mos)	0	
No response (stable)	4 (5–8 wks)	1 (7 mos)	
Progression	34	5	

* Unless otherwise specified, values = No. of patients.

RESULTS

Patient characteristics, treatment results, and toxic ef-

[1]Received Nov 28, 1984; revised Feb 28, 1985; accepted Mar 8, 1985.

[2]Supported in part by Public Health Service grants CA-14028, CA-20319, CA-04920, CA-12644, CA-04915, CA-03389, CA-26766, CA-13612, CA-03096, CA-27057, CA-21116, CA-28862, CA-22433, CA-22411, CA-16385, CA-13238, CA-14028, CA-12014, and CA-32102 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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[8]We thank Liboria Militello for data coordination, and Rosalie Iarussi

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TABLE 2.—Toxic effects*

Toxic effects	Soft tissue sarcomas		Bony sarcomas		Total
	Good-risk	Poor-risk	Good-risk	Poor-risk	
Hematologic					
Wbc count (cells/μl)					
3000–3900	5	0	0	2	7
2000–2999	4	3	2	0	9
1000–1999	7	0	0	1	8
< 1000	4	0	0	0	4
Total	20	3	2	3	28
Platelet count (cells/μl)					
75,000–99,999	3	0	1	0	4
50,000–74,999	3	0	1	1	5
25,000–49,999	5	2	0	0	7
< 25,000	5	0	0	1	6
Total	16	2	2	2	22
Nausea/vomiting	13	5	2	2	22

* Values = No. of patients.

fects are listed in tables 1 and 2. Forty-five of 51 patients were fully evaluable. One partial response (2.2%) was observed in a 41-year-old woman (good-risk) with metastatic leiomyosarcoma of the uterus to lung, lasting 4 months. A 66-year-old man (poor-risk) with metastatic chondrosarcoma to lung had stable disease on AZQ for 7 months. Four additional patients with soft tissue sarcoma had short periods of stability on treatment ranging 5–8 weeks (total stable, 11.1%). All other patients had progressive disease. The major toxic effects were hematologic consisting of leukopenia, thrombocytopenia, and mild nausea and vomiting.

DISCUSSION

Only one partial response and one prolonged period of stable disease were observed in this phase II study of AZQ in previously treated advanced soft tissue and bony sarcomas. Based on these results, we conclude that AZQ, as used in this study, had no significant activity in the treatment of previously treated advanced soft tissue and bony sarcomas.

REFERENCES

1. DRISCOLL JS, DUDECK L, CONGLESTON G, ET AL. Potential CNS antitumor agents VI. Aziridinylbenzoquinones III. J Pharm Sci 68:185–188, 1979.
2. Clinical Brochure. Aziridinylbenzoquinone (NSC 182 986). Bethesda, MD, National Cancer Institute, Aug 1979.
3. TAYLOR S. Minutes of the Phase I Working Group, December, 1981.
4. KRAKOFF I. Minutes of the Phase I Working Group, December 2, 1980.
5. ZIDAR BL, BENJAMIN RS, FRANK J, ET AL. Combination chemotherapy for advanced sarcomas of bone and mesothelioma utilizing rubidazone and DTIC: a Southwest Oncology Group study. Am J Clin Oncol 6:71–74, 1983.

Midwest Regional Oncology Conference II

Kansas City, Missouri, October 17–18, 1985

The goal of this conference is to provide the dietitian, professional nurse, and social worker with current theories and approaches to the treatment and care of the oncology patient. Upon completion of the conference, participants will be able to: (*a*) discuss cancer treatment approaches and outcome, (*b*) identify nutritional interventions and ethical concerns, (*c*) identify psychosocial responses to the diagnosis and treatment of cancer, (*d*) promote standardization of care for the oncology patient, and (*e*) describe effects of legislation on oncology care.

The conference will be held at the Alameda Plaza Hotel and is sponsored by the St. Luke's Hospital of Kansas City. It has been approved for 13 hours by the Missouri Nurses Association, the Kansas Behavioral Sciences Regulatory Board, and the American Dietetic Association. Missouri has reciprocity with most Midwestern states. The fee is $120 for both days or $75 for one day (early registration).

For further information, contact: Theresa Pacitti, Center for Continuing Education, St. Luke's Hospital of Kansas City, Wornall Rd at 44th, Kansas City, MO 64111 (telephone: 816-932-2407).

Current Concepts in Medical Oncology 1985

New York, New York, October 21–25, 1985

This 5-day program is approved for 27 hours of Category I CME credit. The objective is to present the most current information on medical management of patients with cancer. There will be a strong emphasis on the clinical application of information. The course director is George J. Bosl, MD.

For further information, contact: Continuing Medical Education Planning Office, C180, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021 (telephone: 212-794-6754).

Diagnosis and Treatment Strategies for Gynecologic Cancer

Houston, Texas, November 13–16, 1985

This 29th annual Clinical Conference, sponsored by the University of Texas M. D. Anderson Hospital and Tumor Institute, will include comprehensive presentations on the more common endometrial, cervical, and ovarian cancers in addition to such topics as biological response modifier therapy and tumor markers.

For further information, contact: Office of Conference Services, Box 131, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030 (telephone: 713-792-2222).

Management of Cancer Pain

New York, New York, November 14–16, 1985

This 3-day program will grant 14 hours of Category I CME credit. The course will cover pain syndromes, measurement, therapeutic approaches, including use of narcotics, neurosurgical procedures, behavioral/cognitive approaches, and supportive therapy. The course director is Kathleen M. Foley.

For further information, contact: Continuing Medical Education Planning Office, C180, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021 (telehone: 212-794-6754).

Counseling Strategies for Physicians

Oahu, Hawaii, March 24–29, 1986

This conference is designed to introduce the latest techniques and research findings on matters that influence doctor-patient relationships. Faculty will discuss social change, family violence, addiction, and human sexuality as considerations in dealing with patients. They will present problems likely to be

encountered with specific groups of clients such as teenagers, minority groups, aging clients, and pregnant women.

The program is being sponsored by the University of Texas at Tyler, Department of Social Sciences, in cooperation with The University of Texas Health Science Center at Houston, Division of Continuing Education; the cost is $325.00. Seventeen hours of accreditation from the AMA/PRA (Category 1) are approved. The course is also AAFP-prescribed.

For further information, contact: Division of Continuing Education, The University of Texas Health Science Center at Houston, 1100 Holcombe, Rm 1501, Houston, TX 77225 (telephone: Texas—1-800-392-4900-737; outside Texas—1-800-332-8747).

1986 Annual Meeting of the American Radium Society

San Francisco, California, April 6–10, 1986

For further information, contact: Ms. Suzanne Bohn, Executive Secretary, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3179).

First International Congress on Cancer and Hormones

Rome, Italy, April 23–27, 1986

This conference will include such topics as newer diagnostic and therapeutic approaches; epidemiology of endocrine-related cancer; hormone receptors and tumors; oncogenes, growth factors, and small effectors; hormones and cell proliferation; and cancers arising in endocrine glands.

For further information, contact: Stefano Iacobelli, MD, Laboratorio di Endocrinologia Molecolare, Istituto di Clinica Ostetrica e Ginecologica, Università Cattolica del S. Cuore, L.go Agostino Gemelli, 8, 00168 Rome, Italy (telephone: [06] 33.054.496; telex: 611330 UNCATRO).

Fellowship Programs From the International Union Against Cancer (UICC)

The American Cancer Society-Eleanor Roosevelt International Cancer Fellowships are granted normally for 1 year. The closing date for receiving applications is October 1.

The Yamagiwa-Yoshida Memorial International Cancer Study Grants are awarded for periods ranging from 28 to 90 days. Closing dates for receiving applications are June 30 and December 31.

The International Cancer Research Technology Transfer awards are for periods up to 28 days. Applications are reviewed on a continuous basis.

For further information, contact: International Union Against Cancer, Rue du Conseil-Général 3, 1205 Geneva, Switzerland (telephone: [41-22] 20 18 11; telex: 429 724).

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A Publication of the National Cancer Institute

NIH Publication No. 85-22